SOIL SCIENCE

Contents

ERRATA

"Ion exchange in relation to soil acidity," by W. P. Kelley and S. M. Brown

Page 289, footnote 2, should read "The translation was made *jointly* by *S. M. DuToit and R. V. Allison.*"

Review of German literature on soil science and plant physiology in 1925

Pages 213–219. All references to Landw. Vers. Sta. volumes 53 and 54 should read volumes *103* and *104*, respectively.
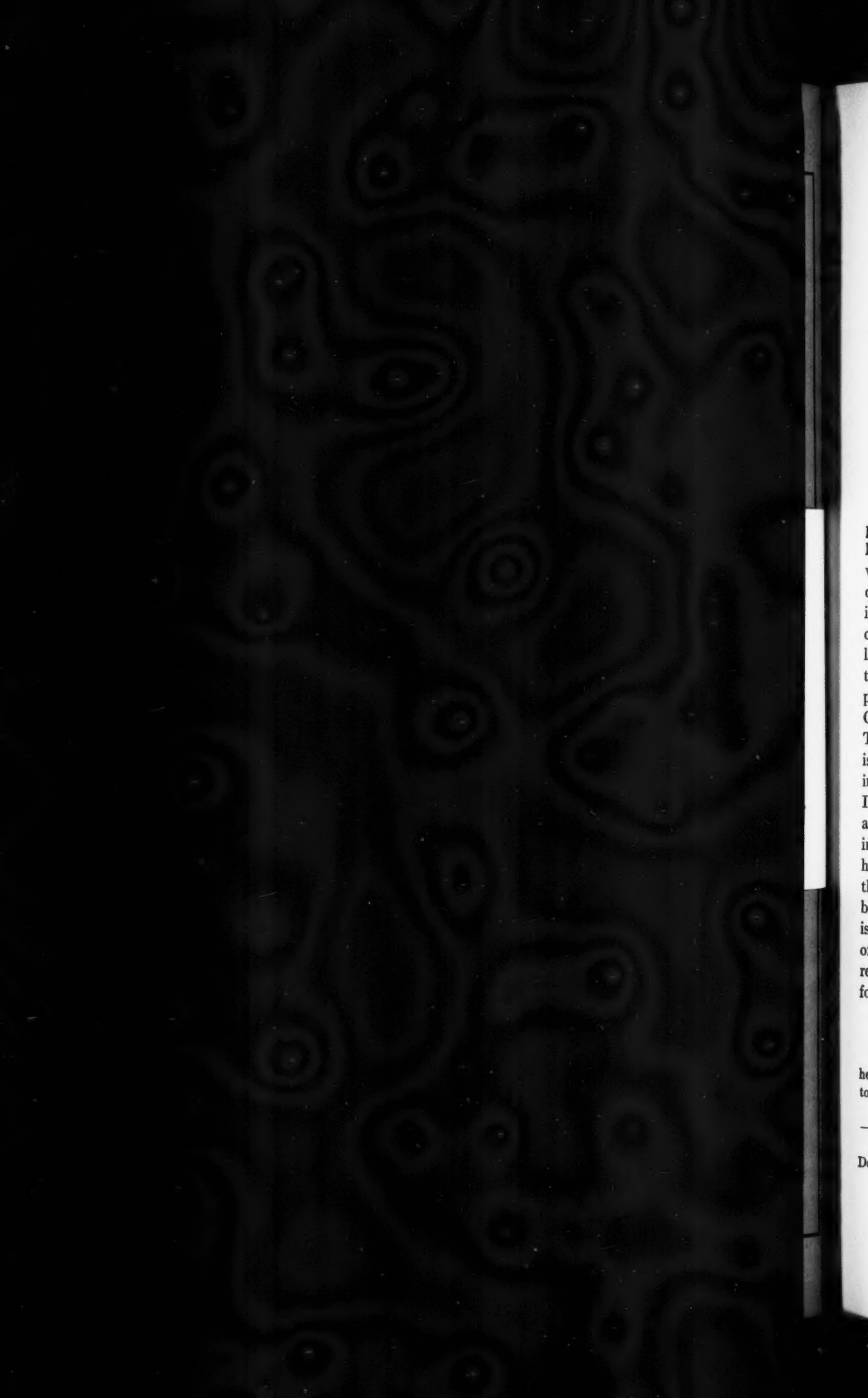
MICROBIOLOGICAL ANALYSIS OF SOILS AS AN INDEX OF SOIL FERTILITY: X. THE CATALYTIC POWER OF THE SOIL[1]

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INTRODUCTORY

The catalytic power of the soil, or the ability of soils to decompose hydrogen peroxide with the liberation of molecular oxygen, has received considerably less attention than any of the other soil microbiological processes studied previously (37, 38). As a matter of fact, the catalytic action of the soil is frequently not considered a microbiological process, but is ascribed to the various inorganic soil constituents and to the soil colloids (10). The origin of the catalytic power of the soil, however, is considered by some investigators to be largely a result of microbiological activities. Not only is catalase contained in the bodies of many microörganisms developing in the soil, but this enzyme is present in the various vegetable manures and plant residues added to the soil. Certain mineral constituents of the soil also exert a definite catalytic action. The addition of manure to the soil leads to a rapid development of microörganisms; however, it is still questionable whether this is accompanied by an increase in the ability of the soil to liberate oxygen from hydrogen peroxide. In winter, both the development of microörganisms in the soil and its catalytic action diminish rapidly, whereas in the warmer months of April to June, both increase again. König (14) observed that soils in which the microörganisms have been killed by steam or antiseptics possess a much lower catalytic action than untreated soils. If the catalase found in different soils is largely of microbiological origin and indicates present or past activities of microörganisms, one is justified in considering the catalytic activity of soil from the point of view of microbiological activities, in an attempt to learn whether there is any correlation between microbiological activities and soil fertility and whether the former can serve as an index of the latter.

HISTORICAL

The literature dealing with the subject of catalase has been reviewed in detail by Oppenheimer (25), Battelli and Stern (3), and Morgulis (22). It is sufficient to call attention here to those contributions which have a direct bearing upon the problem under consideration.

It has been known since the work of Thénard (35) that plant and vegetable tissues are

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capable of decomposing hydrogen peroxide into water and molecular oxygen. Berzelius (4) and Schoenbein (29a) seem to have been the first to mention that soils possess a catalytic power. Impressed by the fact that catalase is found abundantly in the cells of bacteria and fungi, which are of common occurrence in the soil, Loew (17) suspected that the soil should contain also an abundance of catalase. This was confirmed by actual observation. It was König and his associates (12-15), however, who carried out a systematic series of investigations on this subject, and who showed that soils differ greatly in their catalytic action and that this property is due largely to the enzyme catalase.

Loew (17) differentiated between two different catalases: (a) β-catalase, soluble in cold water, and (b) α-catalase, insoluble in cold water but extracted by treatment for 15 hours with 0.2 per cent sodium hydroxide solution or for 1 hour with a sodium carbonate solution, at 30°. An aqueous solution free from bacteria was found to lose the β-catalase rapidly, as a result of auto-oxidation or some intramolecular change. Nitrates seemed to depress materially the action of the enzyme, without injuring the enzyme itself. Potassium salts retarded the reaction more than sodium salts; sodium carbonate, however, markedly stimulated the reaction. Acids were generally found to be injurious to the enzyme.

According to Sörensen (31), catalase has its optimum at neutrality (pH 7.0). Michaelis and Pechstein (20), however, claimed that the action of catalase increases by increasing alkalinity up to pH 9.0; Sörensen's results were ascribed to the toxic action of phosphates, which were used as buffers, the former using acetates and carbonates as buffering agents. Both Bodansky (5) and Morgulis (21) noted that the optimum reaction of catalase is at pH 7.0 to 7.5, the action decreasing rapidly on the acid side of the optimum but not on the alkaline. Stapp (32) found the optimum for the action of bacterial catalase to be at pH 7.5 to 8, with a drop on both sides of the optimum. Different acids vary in their toxicity, as shown by the following series (29): $HNO_3 > H_3PO_4 > H_2SO_4 > HCl$. The injurious action of the anions depends largely on the reaction of the medium, being very pronounced at pH 3.0 to 5.0 and hardly noticeable at pH 7.3 to 8.0 (27). It is interesting to note that Bach and Oparin (1) found that the addition of lime to the soil increases greatly the catalytic action of germinating seed.

Heating a bacterial suspension at 80°C. for 15 minutes is sufficient to inactivate completely the action of catalase; the spores will still continue to contain some catalase, even when heated at 100°C. Treatment of a bacterial culture with chloroform and acetone may even stimulate its catalytic action but prolonged treatment will weaken it. In general, the resistance of bacterial catalase to disinfectants is distinct from that of the bacterial cells themselves (32).

The physiological rôle of catalase in the growth of an organism or a cell has been made the subject of numerous investigations. It is sufficient to mention the suggestion that oxidation processes in the organism or in the cell result in the formation of H_2O_2, which would become injurious were not the peroxide immediately destroyed by the catalase formed by the cells. Anaerobic organisms possess only a slight catalytic action and are rapidly injured by the addition of a small amount of hydrogen peroxide (9). Rywosch (28) found that although low concentrations of H_2O_2 do not injure aerobic strains of B. coli, they are sufficient to kill rapidly the anaerobic strains of this organism. According to McLeod and Gordon (19), the inability of anaerobes to produce catalase, limits their growth in the absence of air. Kluyver (11) formulated the theory that those organisms which obtain their energy by the use of elementary oxygen contain catalase, whereas those that utilize the energy liberated by oxidation-reduction (fermentation) processes do not form catalase. Callow (6) tested nine anaerobic bacteria and twelve aerobic species; none of the anaerobes were found capable of producing catalase, whereas every aerobic form, except the streptococci, produced catalase. Stapp (32) found that both bacteria and yeast cultivated under anaerobic conditions were poorer in catalase than when grown in the presence of free oxygen. Virtanen and Karstrow (36), however, found that facultative anaerobic bacteria are richer in catalase when grown anaerobically, perhaps because the catalase is thus not used up for the decomposition of H_2O_2, which

is formed under aerobic conditions. Attention will be called later to the bearing that this phenomenon may have upon the catalytic power of the soil.

It is important to recall here the observation made by Morgulis and Levine (23) that benzol and a number of its homologues and derivatives act upon hydrogen peroxide in a manner similar to catalase. The decomposition of the peroxide is very violent, the maximum of the reaction being immediately attained and the reaction itself coming rapidly to a standstill. The action of these substances is thus distinctly different from that of catalase and of inorganic catalysts, which obey the laws of enzymatic and other catalyzed reactions. Similar observations have been made by Wu (41) on the catalytic action of hematin and all iron-containing derivatives of hemoglobin.

The contributions to the subject of the catalytic action of soils are meagre and, with very few exceptions, rather inconclusive. König and associates (12) reported that the catalytic action of soils is due to the action both of catalase and of inorganic catalysts, as shown in the following summary:

Oxygen given off from 20 cc. of 2 per cent H_2O_2 solution by 5 gm. of air-dried soil

SOIL TREATMENT	INCUBATION	SANDY SOIL	LOAM SOIL	CALCAREOUS SOIL	CLAY SOIL
	minutes	cc.	cc.	cc.	cc.
Untreated.................	15	2	13	38	24
	60	4	32	80	51
	120	6	43	103	67
Ignited...................	15	...	1	2	1
	60	0.5	7	12	4
	120	0.5	16	26.5	10
Autoclaved 3 hours at 3 atmospheres.	60	3.0	13.5	23	21
Autoclaved 3 hours at 7 atmospheres.	60	5.0	15.5	13	26
Treated with chloroform..........	15	...	5	12	12
	60	...	11	15	18
	120	0.5	14	17.5	20
Treated with HCN...............	15	...	..	2	..
	60	...	6	7	1
	120	...	12	13	6

Ignited and autoclaved soils still continue to decompose H_2O_2 because of the presence of sesquioxides. König and his associates (13) found that manganese and iron oxides are most active, whereas calcium and aluminum oxides are less active, 5 gm. of CaO liberating 20 cc. of oxygen in 1 hour, and 2 gm. of Mn_3O_4 or Fe_2O_3 liberating 140 cc. in 5 minutes. Since, however, these oxides are present in the soil in inconsiderable amounts, their action is probably negligible, May and Gile (18) having found that a clay subsoil of Porto Rico containing 29.3 per cent of ferric and aluminum oxides was almost devoid of catalytic action.

There seems to be, however, a definite correlation between the content of organic matter in the soil and its catalytic action, as shown by the following comparison:

Catalytic power of the soil in relation to its "humus" content

	SANDY SOIL	LOAM SOIL	CALCAREOUS SOIL	CLAY SOIL
"Humus".....................	100	203	453	198
Oxygen liberated in 2 hours.......	100	721	1,721	1,117

The considerably greater increase of the catalytic power over that of the "humus" content is due to the presence of iron and manganese and other oxides in the heavier and calcareous soils.   Similar results were obtained by Balks (2).   Osuga (25) demonstrated that ferric oxide, manganese oxide, and "humus" show marked catalytic action; he suggested that these substances may be the main constituents which impart to the soil its distinct action upon hydrogen peroxide; he believed that the influence of bacteria upon soil catalysis was rather small, thus confirming the previous observations of Kappen (10).   Stoklasa and associates (33) reached conclusions similar to those of König; the catalytic power of a soil containing the same amount of iron and manganese compounds increases with an increase in the biochemical activities, an increase in the fineness of soil structure and in alkalinity.   Smolik (30) also attempted to find a correlation between the physical properties of the soil and its catalytic action; his results are, however, far from conclusive.

May and Gile (18) ascribed the catalytic action of soils to the presence of microörganisms and to their activities, especially in the decomposition of organic matter.   Soils in which these activities were greatest were most active catalytically.   The action of inorganic soil colloids was found to be negligible in comparison with the action of the enzyme.   May and Gile suggested that the time required to bring about the evolution of a certain volume of oxygen from a certain quantity and concentration of peroxide should be used as a measure of the catalytic action of the soil.   The addition of manures and fertilizers, however, did not seem to affect the activity and content of catalase in the soil even after 3 months.   Sullivan and Reid (34) reported that the catalytic action of surface soils is greater than that of subsoils and that of fertile soils greater than that of infertile soils.

Chouchack (7) suggested a novel method of determining by its catalytic action the need of certain fertilizing elements in the soil.   If a soil is deficient in a certain element it will respond to the application of that element by an increase in bacterial activity; the latter will manifest itself by an increase in the catalytic power of the soil, which can be used as a "biological index" of the need of soil for the elements essential for the growth of higher plants.   This index was defined as the difference between the amount of oxygen liberated from a given amount of H_2O_2 solution under a definite set of conditions by 4 gm. of soil before and after boiling for 1 minute.   A series of samples of the soil in question receive an application of mannite and of the various fertilizing elements; moisture is then added to bring them to an optimum and the soils are incubated at 28°C. for 24 hours.   Four-gram samples of the soil are then used for the catalytic test, which lasts for 15 minutes.   The results seem to point to a parallelism between the change in the catalase content and the response in crop yield, as a result of the application of the various fertilizers.   Chouchack (7) pointed out the advantage of this method over field experiments for determining the fertilizer requirements of the soil.

EXPERIMENTAL

The action of catalase is usually determined by adding a definite amount of soil to a certain quantity of hydrogen peroxide solution, adjusted to neutrality. The mixture is incubated at a definite temperature for a definite length of time and either (a) the residual peroxide is determined by titrating with $KMnO_4$, or (b) the amount of oxygen gas evolved in the reaction is measured.   In some cases the time required for the evolution of a definite amount of oxygen, under standard conditions, is used as a measure.   Morgulis (21) and Northrup (24), however, have shown that the concentration of the enzyme rapidly decreases with the time of action, and that the rate of the reaction is independent of the initial concentration of the peroxide.   In the case of soil catalase, the problem is much more complex than in the case of animal or plant catalase, because of

the disturbing influence of the action of organic and inorganic complexes, non-enzymatic in nature. It is, therefore, best to limit the test to a definite set of conditions. In the following experiments, 5-gm. portions of air-dry soil were added to 20 cc. of a 1.5 per cent solution of H_2O_2, previously neutralized and warmed to 37°C.; the mixture was shaken 10 times and placed in a thermostat for 20 minutes at 37°C. Readings were taken at the end of 10 and 20 minutes. The gas is collected in burettes over a 2 per cent NaOH solution, so as to absorb the CO_2 liberated in the process.

TABLE 1

Influence of various buffering agents upon the catalytic action of the soil

SOIL TYPE	NATURE OF DILUENT*	OXYGEN GIVEN OFF IN 10 MINUTES
		cc.
Unlimed, acid soil........................	Water	15.1
	Phosphate mixture	17.0
	Water	22.6
	Phosphate mixture	17.9
Same soil, limed........................	Citrate mixture	24.3
	Acetate mixture	24.4
	Clark's solution	18.6

* Reaction of acid soil, pH 5.2; of limed soil, pH 6.5. Sufficient buffer solution added to change the reaction of the soil to about pH 6.8–7.0.

TABLE 2

Influence of heat upon the catalytic action of the soil

SOIL HEATED	OXYGEN EVOLVED AFTER	
	10 minutes	20 minutes
	cc.	*cc.*
Control..	15.5	22.8
50°C., 1 minute...................................	8.3	14.4
75°C., 1 minute...................................	7.2	12.2
100°C., 1 minute..................................	5.0	8.1
15 pounds pressure, 15 minutes....................	0.5	0.7

Since it has been found that the reaction of the soil greatly influences the action of catalase, it was thought advisable to test first the effect of buffers upon the catalytic action of the soil. The results are given in table 1.

The use of the phosphate mixture at pH 7.0, with an acid soil of pH 4.8, resulted in a slight increase in its catalytic action, but the addition of phosphates to the limed soil resulted in an injurious effect upon the catalytic action. The injurious effect of the phosphate upon the catalytic action of the acid soil is more than balanced by the favorable effect exerted by making the soil

less acid. The use of acetates and citrates as buffers gave more favorable results.

To determine the influence of heat in the destruction of the catalase, so as to differentiate between the catalytic power of the soil due to the presence of the enzyme catalase and the action due to the inorganic substances of the soil capable of decomposing hydrogen peroxide, 5-gm. portions of soil were treated with 10-cc. portions of water and heated at different temperatures; 10-cc. portions of 3 per cent H_2O_2 solution were then added and the oxygen liberated was determined. (Table 2.) Heating the moist soil for 1 minute was not sufficient to destroy all the catalase in the soil. It is actually necessary to autoclave the soil before the action of the catalase is destroyed.

TABLE 3

Influence of reaction upon the catalytic power of the soil

TREATMENT OF 50 GM. OF SOIL	AFTER 2 DAYS					AFTER 15 DAYS		
	pH	Oxygen given off				pH	Oxygen given off	
		Before autoclaving		After autoclaving			10 minutes	20 minutes
		10 minutes	20 minutes	10 minutes	20 minutes			
		cc.	cc.	cc.	cc.		cc.	cc.
0.5N H₂SO₄, 10 cc..........	3.3	5.2	9.2	4.6	7.7	3.7	3.2	3.7
0.5N H₂SO₄, 5 cc..........	3.4	9.5	14.0	2.1	3.6	4.1	4.7	6.6
0.5N H₂SO₄, 2.5 cc..........	3.8	11.8	16.9	....	....	4.4	6.8	9.0
0.5N H₂SO₄, 1.0 cc..........	4.4	16.9	26.7	....	....	4.8	8.6	9.3
None......................	5.0	20.0	32.3	1.1	2.7	5.0	15.9	19.0
CaO, 10 mgm..............	5.2	24.4	37.2	3.1	6.1	5.0	24.0	28.7
CaO, 20 mgm..............	5.3	27.2	40.9	3.2	6.2	5.1	16.6	21.1
CaO, 50 mgm..............	5.5	31.3	43.6	7.3	13.4	5.2	18.2	30.9
CaO, 100 mgm.............	5.8	32.8	45.7	7.5	14.6	...	....	....
CaO, 200 mgm.............	6.5	47.1	>50.0	12.5	19.0	...	....	....
CaO, 500 mgm.............	8.2	>50.0		19.6	29.2	...	....	....

Since the catalytic action of the soil was found to depend not only upon the presence of catalase and of certain inorganic soil constituents, but also upon the soil reaction, it was essential to find out how a change in the soil reaction influences the catalytic action of the soil as a whole and that of the enzyme catalase present in the soil. For this purpose, varying quantities of sulfuric acid and of CaO were added to 50-gm. portions of soil and the mixture was allowed to stand for 2 and 15 days, when the catalytic action was determined. (Table 3.) These results definitely indicate that increasing acidity rapidly depresses the action of catalase, whereas decreasing acidity stimulates the action of the enzyme. CaO itself acts catalytically, as seen by the gradual increase in catalytic action of the autoclaved soil. Subtracting the values obtained for the autoclaved

soil from those of the unautoclaved soil, however, shows that the "biological index" constantly increases with an increase in the pH value of the soil. Prolonged standing of the soil leads to a diminution in the catalytic action; this may be due to the fact that air-dried soil has been used for this experiment. Moistening of this soil brought an increase in the biological activities, whereas continued incubation of the moist soil led to a reduction of these activities.

Further information on the influence of hydrogen- and hydroxyl-ion concentration upon the catalytic action of the soil was obtained by heating black alkali soil (pH 9.6) with different portions of H_2SO_4, in order to adjust the reaction to different pH values; the catalytic action of the various soil portions was determined after 2 and 15 days. (Table 4.) A large part of the catalytic action of the untreated soil is thus found to be due to the inorganic soil constituents, namely the cations, as shown by the small decrease in action, as a result of autoclaving the soil. An increase in the hydrogen-ion concentration

TABLE 4

Influence of reaction of a "black alkali soil" upon its catalytic action

FINAL REACTION	OXYGEN GIVEN OFF					
	After 2 days		After 15 days		After autoclaving	
	10 minutes	20 minutes	10 minutes	20 minutes	10 minutes	20 minutes
pH	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
9.6*	31.5	43.3	....	....	20.3	32.7
8.0	....	....	20.8	32.4	10.2	19.6
7.2	16.5	21.1	6.7	11.0	7.4	14.5
6.2	4.9	6.2	2.5	5.4	1.0	1.8
3.6	0	0	0.3	1.2	0.5	0.6

* Control.

leads to a depression of the action of these inorganic catalytists. The same result can be accomplished not only by acids but also by appropriate buffering agents.

In order to determine how catalase may be actually formed in the soil, different substances were added under various conditions, so as to bring about an increased development of various groups of organisms in the soil. In the first experiment, 10-gm. portions of cellulose in the form of ground filter paper were added to a series of 1-kgm. portions of soil placed in pots; various amounts of water were then added so as to bring the moisture content of the soil to 30 per cent of its moisture-holding capacity, to 60 per cent, and to full saturation (about 35 per cent of soil weight). After 1 month incubation, the soils were tested for their catalytic action. (Table 5.)

The addition of cellulose to the soil was found to bring about only a slight increase in the catalytic power of the soil. An increase in moisture content led

to a decrease of the ability of the soil to liberate oxygen from H_2O_2, and ammonium sulfate also had a somewhat depressing effect, especially with a low moisture content. This experiment was repeated with practically identical results

TABLE 5

Influence of cellulose decomposition upon the catalytic action of the soil at different moisture contents

CELLULOSE USED	NITROGEN SOURCE	MOISTURE	OXYGEN GIVEN OFF IN	
			10 minutes	20 minutes
			cc.	*cc.*
None.........	None	10 per cent	26.8	41.9
		20 per cent	20.9	33.7
		Saturated	14.8	25.7
	Ammonium sulfate	10 per cent	20.0	30.8
		20 per cent	16.2	26.5
		Saturated	13.5	22.1
1 per cent.....	None	10 per cent	29.5	42.9
		20 per cent	22.9	35.2
		Saturated	17.4	26.9
	Ammonium sulfate	10 per cent	29.2	39.0
		20 per cent	15.7	26.9
		Saturated	19.3	29.4

TABLE 6

*Influence of cellulose and inorganic nitrogen compounds upon the catalytic action of the soil**

CELLULOSE USED	NITROGEN SOURCE	OXYGEN GIVEN OFF IN	
		10 minutes	20 minutes
		cc.	*cc.*
None............	None	17.0	22.3
	Ammonium sulfate	16.5	22.0
	Sodium nitrate	16.3	21.5
1 per cent........	None	18.4	24.2
	Ammonium sulfate	17.9	24.4
	Sodium nitrate	20.0	26.3

* Moisture content—25 per cent.

as far as cellulose is concerned. There was a less marked injury as a result of an addition of nitrogen salts, perhaps because of the relatively high moisture content. (Table 6.) The low catalytic power resulting from the addition of celluloses may be due to the fact that fungi are largely concerned in the decom-

position of celluloses in the soil (40) and to the fact, as shown by Dox (8), that many fungi produce only a very small amount of catalase.

It was pointed out above that Chouchack (7) found that the addition of

TABLE 7

Influence of mannite, buffer mixture and mineral elements upon the catalytic action of a soil

			PERIOD OF INCUBATION					
			2 days		18 days		49 days	
SOIL TYPE		TREATMENT OF SOIL	Oxygen given off in		Oxygen given off in		Oxygen given off in	
			10 minutes	20 minutes	10 minutes	20 minutes	10 minutes	20 minutes
			cc.	cc.	cc.	cc.	cc.	cc.
Acid soil	1	Dry soil	11.6	17.4	....	....	....	....
	2	Moist soil	12.0	18.3	14.1	19.2	....	....
	3	Soil + mannite	23.9	28.1	9.9	13.8	7.3	9.5
	4	Soil + mannite + 0.1 per cent $(NH_4)_2SO_4$	15.3	18.9	15.3	19.3	12.0	15.4
	5	Soil + 0.1 per cent $(NH_4)_2SO_4$	16.5	24.0	9.2	13.0	7.0	11.0
	6	Soil + mannite + 0.1 per cent $Ca(H_2PO_4)_2$	19.1	22.8	13.0	17.1	8.0	12.0
	7	Soil + mannite + 0.1 per cent K_2SO_4	15.8	18.1	....	17.8	10.2	13.5
	8	Soil + 0.5 per cent $CaCO_3$	....	48.6	19.7	26.8	....	18.2
	9	Soil + 2 cc. phosphate mixture	15.0	19.7	17.0	21.3	17.0	20.0
	10	Soil + 2 cc. sodium citrate-citric acid mixture	38.8	43.5	43.0	45.5	21.0	26.2
Limed soil	1	Dry soil	11.7	18.3	....	....	10.5	16.7
	2	Moist soil	12.8	18.9	14.3	19.4	....	....
	3	Soil + mannite	36.3	40.6	24.8	30.5	20.7	25.0
	4	Soil + mannite + 0.1 per cent $(NH_4)_2SO_4$	57.7	59.3	29.3	33.2	24.4	28.8
	5	Soil + 0.1 per cent $(NH_4)_2SO_4$	18.8	28.0	12.7	17.7	12.7	16.8
	6	Soil + mannite + 0.1 per cent $Ca(H_2PO_4)_2$	34.5	38.2	24.5	28.5	17.5	21.6
	7	Soil + mannite + 0.1 per cent K_2SO_4	57.3	60.0	33.5	37.7	23.6	28.0
	8	Soil + 0.5 per cent $CaCO_3$	20.2	28.0	15.9	21.3	14.1	18.9
	9	Soil + 2 cc. phosphate mixture	9.9	14.1	15.0	18.4	10.8	13.1
	10	Soil + 2 cc. sodium citrate-citric acid mixture	55.7	60.5	28.6	36.5	18.7	23.8

mannite to the soil greatly stimulates its catalytic power, especially in the presence of minerals in which the soil is lacking. An experiment was, therefore, instituted to throw some light upon the influence of the addition of mannite upon the catalytic action of the soil, using an acid soil (A, pH 4.8) and a limed

soil (B, pH 7.0); 1 per cent mannite and 0.1 per cent portions of different minerals were added to a series of 100-gm. portions of soil. The optimum amount of moisture was then added, the soils were placed in covered tumblers and incubated at different intervals; 5-gram portions of soil were taken out and tested for the catalytic action. (Table 7.)

The results indicate very definitely that the addition of mannite brings about a decided increase in the catalytic action of the soil. This action is further increased, in the case of the limed soil, by the addition of nitrogen and mineral elements. On the other hand, the addition of $CaCO_3$ increases the catalytic action of the acid soil, but only slightly that of the limed soil. It is interesting

TABLE 8

The catalytic action of cranberry peat soils

		OXYGEN GIVEN OFF BY 5 GM. OF SOIL			
		Untreated soil		Autoclaved soil	
NATURE OF SOIL	pH VALUE	10 minutes	20 minutes	10 minutes	20 minutes
		cc.	cc.	cc.	cc.
Unlimed bog....................................	4.0	38.5	47.1	1.5	3.2
3000 pounds of $CaCO_3$ per acre annually for last 3 years..	7.1	25.2	32.2	1.6	3.2
Old bog, turfed.................................	3.7	35.1	44.6	12.4	30.2
Old bog, unturfed..............................	3.7	41.0	54.7	3.4	16.0
New bog, dark colored.........................	3.8	45.3	57.3	15.8	19.3
New bog, brown	3.7	42.1	55.0	6.0	12.5
Plot with water table 18 inches below surface, 1–3 inches depth................................	3.6	53.2	83.9	2.3	3.9
Plot with water table 18 inches below surface, 10–12 inch depth....................................	3.4	4.1	4.1	0.2	1.3
Plot with water table at surface...................	3.5	2.5	4.3	0.9	2.5
"Humus" from forest soil........................	....	39.4	41.3	....	....
0.5 gm. of mixed dry fungus mycelium............	....	22.0	24.2	....	....

to compare the rapidity of the decomposition of the hydrogen peroxide by the soil to which mannite has been added with the action of the fungus mycelium and the acid peat and forest soils (table 8). This action is similar to that of the organic ring compounds investigated by Morgulis and Levine (23) and of the compounds studied by Wu (40). Perhaps we are not dealing here with an actual increase of catalase in the soil, but merely with the formation of certain organic compounds, either directly from the mannite or from the microbial protoplasm. The greater increase of the catalytic reaction in the limed than in the acid soil, as a result of an addition of mannite, is probably due to the fact that many bacteria (Azotobacter, etc.) would be capable of developing in the former and not in the latter soil.

The addition of phosphate mixture brought about a slight increase in the catalytic action of the acid soil but had a depressing effect on the limed soil. The addition of the citrate mixture increased the catalytic action of the acid soil even more markedly than that of the limed soil, perhaps partly because the citrate was used as a source of energy by the microörganisms.

Several cranberry peat soils from South Jersey were used (table 8) as a source of further information on the catalytic action of soils rich in organic compounds. To give 1 gm. of dry material, 5 to 20 gm. of soil was required, indicating the large amount of water that these soils can hold. The interesting result is that with a high water table, the catalase is absent, which may be accounted for by the fact that anaerobic organisms do not produce any

TABLE 9

Influence of soil treatment upon crop yield and catalytic power of the soil

PLOT NUMBER	SOIL TREATMENT	TESTED JULY 20			TESTED SEPTEMBER 14			YIELD OF WHEAT PER ACRE, IN 1925	
		Oxygen given off in		Order	Oxygen given off in		Order		
		10 min-utes	20 min-utes		10 min-utes	20 min-utes		Pounds	Order
		cc.	*cc.*		*cc.*	*cc.*			
5A	Manure + minerals	21.2	27.1	1	17.1	23.1	1	7,251	2
7A	Nothing	8.9	11.8	10	10.6	14.4	8	393	12
9A	Minerals + NaNO₃	14.2	18.3	4	12.1	16.4	4	6,263	4
11A	Minerals + (NH₄)₂SO₄	3.1	4.1	12	5.5	7.6	12	451	11
17A	Minerals + straw	8.4	12.8	8	12.8	16.0	5	4,145	7
18A	Minerals + manure + NaNO₃	16.6	20.5	3	15.0	17.4	3	8,238	1
19A	Minerals only	7.7	10.1	11	10.6	12.5	11	3,141	9
4B	Minerals + lime	11.2	14.5	7	10.6	14.0	9	3,175	8
5B	Minerals + manure + lime	17.3	21.5	2	14.4	18.8	2	4,973	5
7B	Lime only	13.9	17.1	5	10.8	15.7	6	2,846	10
11B	Minerals + (NH₄)₂SO₄ + lime	7.8	12.2	9	9.7	13.2	10	6,503	3
19B	Minerals only	11.8	16.1	6	11.3	15.3	7	4,669	6

catalase or that the enzyme is destroyed when the soil is saturated with water.

It is rather difficult to explain these results, unless the catalase content of the plants and the nature of the organisms contributing to the decomposition of these plants are known. It is interesting to note the explosive character of the "humus" forest soil and of the fungus mycelium.

The nature of the catalytic action of the soil is very complex because of (a) the inorganic constituents of the soil, which seem to be especially active in alkaline soils; (b) the organic constituents, especially abundant in forest soils and in other soils rich in organic matter; and (c) the enzyme catalase, the amount of which depends on the nature of the plant residues and on the

microörganisms active in the decomposition of these plant residues; as well as on (d) the reaction and moisture content of the soil. The results obtained by the application of such a method would, therefore, be too complex to be used as a basis of comparison among different soils. An attempt was made, however, to use this index for determining the biological condition of a series of soils from various plots under a definite system of fertilizer treatment for the last 18 years, as reported by Lipman and Blair (26) and in the previous papers in this series (37–39). The results are given in table 9.

The limed plots show a considerably higher catalytic power than the unlimed plots. This would be expected from the previous observations on the influence of reaction upon the catalytic action of the soil. These results are also comparable with those reported elsewhere on the numbers of microörganisms and their activities in the soil. Plot 5B is an exception, since it has a high catalytic power and gave a comparatively low crop yield; however, when the crop yields of this plot for the previous years are considered, it is found to be one of the highest yielding plots. The yields are recorded as dry matter, including both straw and grain. Plot 5B gave a considerably greater proportion of grain to straw than plot 18A, for example. Plot 11B, receiving ammonium sulfate and lime, gave a very high yield but a low catalytic power, because of its comparatively acid reaction, which acts injuriously upon the catalase. With these two exceptions, which can be readily explained, the parallelism between the catalytic power and the crop yields is rather interesting.

SUMMARY

1. The liberation of oxygen from hydrogen peroxide by soil is a result of three distinct processes:

(a) The action of the enzyme catalase, which may be either of plant or microbial origin, the latter usually predominating.
(b) The catalytic action of certain organic substances which seem to be found in great abundance in dead fungus mycelium and in soil rich in organic matter.
(c) The action of inorganic catalysts, especially in alkaline soils.

2. An acid reaction is unfavorable to the catalytic action of the soil, whereas a neutral or slightly alkaline reaction is favorable. One must differentiate of course between the influence of the hydrogen-ion concentration of the soil upon the actual amount of catalytic agent formed or present in the soil and upon the course of action of this agent. A high moisture content is injurious to the catalytic action of the soil.

3. There is a certain parallelism between the crop-producing capacity of a soil and its catalytic action. It is doubtful, however, whether this method can be considered as giving information upon microbiological processes in the soil, since frequently the catalytic action is due not so much to the microörganisms of the soil, as to certain inorganic or organic constituents which may be present in abundance in certain soils.

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ON THE ORIGIN AND NATURE OF THE SOIL ORGANIC MATTER OR SOIL "HUMUS": V. THE ROLE OF MICROÖRGANISMS IN THE FORMATION OF "HUMUS" IN THE SOIL[1]

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The previous investigations seem to point to the fact that when organic matter is added to the soil, the soluble carbohydrates, proteins, pentosans and other hemicelluloses, as well as the true celluloses are readily decomposed, leaving the lignins (and probably also some of the fats and waxes) to accumulate in the soil. The assumption that lignin is the only mother substance of the soil "humus" is hardly sufficient, however, to account for the nature of this "humus," especially since the latter is usually found to contain 2.0 to 3.5 per cent nitrogen, whereas pure lignin is free from nitrogen. The preparations of lignin, obtained both by treatment with the concentrated acid and by extraction with sodium hydroxide, however, seem to contain a small amount of nitrogen. The nitrogen part of the "humus" could originate either from the proteins of the organic matter added to the soil or through the synthesizing activities of microörganisms. The former could hardly account for such a large content of nitrogen: first, because most of the natural organic substances contain only a small amount of nitrogen (18 to 20 per cent of lignin and only 0.4 to 0.5 per cent of nitrogen in straw), and secondly, the addition of proteins and of amino acids to the soil greatly stimulates the activities of various groups of microörganisms and results in the rapid decomposition of the proteins with the formation of ammonia. Lathrop (2) and others called attention to the fact that when proteins are added to the soil, they are decomposed but there is, at the same time, a formation of a new protein material which is more resistant to decomposition. This protein material could only be of microbial origin. The idea of Maillard and others (3) that "humus" originates from the chemical interaction of sugars and amino acids can hardly explain this process, since there is at no time in normal soil more than mere traces either of sugars or of amino acids, both of which are rapidly used up by different groups of microorganisms.

It has been pointed out previously that the decomposition of non-nitrogenous substances by microörganisms in the soil leads to an extensive synthesis of new microbial protoplasm, as indicated by the abundant assimilation of inor-

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ganic nitrogen and its transformation into microbial proteins and other complex nitrogenous substances.   If fungi are the active agents of decomposition, 1 part of nitrogen will be assimilated for every 7 to 8 parts of carbon given off as CO_2, in the process of energy utilization.   If pure cultures of bacteria are the active agents in the decomposition of celluloses and other non-nitrogenous substances, 1 part of nitrogen will be assimilated for 12.5 parts of carbon liberated as CO_2.   Since bacterial cells are richer in nitrogen than fungus mycelium, a much smaller amount of protoplasm will be synthesized.   If the mixed soil flora and fauna take part in the active processes of decomposition of the organic matter added to the soil, 1 part of nitrogen will be transformed from an inorganic into an organic form for every 16 to 18 parts of carbon given off as CO_2.

The last process should be the one to be emphasized most, since it considers the soil flora and fauna as a whole, unmodified.   It may be assumed that, in the process of decomposition of plant stubble and straw, 60 to 80 per cent of the carbon is decomposed by the soil organisms, in a relatively short period of time, leaving 20 to 40 per cent of the carbon, which comprises the lignins and some of the fats and waxes, to persist in the soil for a longer period of time.   As a result of decomposition of the 60 to 80 per cent of the carbon, about 40 to 70 per cent is given off as CO_2 and 10 to 20 per cent left in the soil in the form of microbial protoplasm.   This can be readily checked up by another process of reasoning.   If, out of 100 units of carbon added to the soil in the form of natural plant residues, 40 to 70 units are given off as CO_2 and if 1 unit of nitrogen is assimilated by the organisms for every 16 to 18 units of carbon given as CO_2, 2.5 to 4 units of nitrogen will be assimilated.   Since the microbial protoplasm contains 5 to 10 times as much carbon as nitrogen, the assimilation of 2.5 to 4 units of nitrogen will necessitate the assimilation of 10 to 40 units of carbon—probably on the average, 10 to 20 units—since the mycelium of the fungi and the cells of the bacteria, which are the agents assimilating considerable quantities of carbon, would soon be partly decomposed by the other bacteria and actinomyces, liberating a part of the carbon of the mycelium as CO_2. This CO_2 is included already in the total amount considered above for the complex soil population.

These calculations show that very considerable quantities of organic matter—as much as 10 to 20 per cent of the total amount introduced into the soil—are synthesized in the soil by microörganisms.   This synthesized organic matter is very rich in nitrogen, containing as much as 5 to 10 per cent, and is no doubt sooner or later decomposed by the various representatives of the soil flora and fauna.   A part is changed into CO_2, NH_3, etc.; a part is utilized by other organisms and changed into fresh microbial protoplasm; and a part, being resistant to decomposition, is left in the soil.   This last part contributes to the soil organic matter or "humus" and is of great interest in connection with these studies on the origin of "humus" in the soil.   A series of preliminary experiments were undertaken with this idea in mind.

Experiment 1. The formation of "humus" in sand media

The first experiment on the synthesis of "humus" was carried out using sand as a medium, so that the large quantities of organic matter commonly found in the soil should not interfere in the attempt to establish how much "humus" is actually synthesized.   Eight-hundred-gram portions of washed white sand were placed in a series of small earthenware pots.   These were divided into 3 groups: one group of pots received 2 per cent of rye straw, then, after 35 days, again 2 per cent of straw; the second group received also the two treatments of straw on the same respective dates and, in addition, 2 gm. of $(NH_4)_2HPO_4$, 0.5 gm. $MgSO_4$, and 0.5 gm. KCl per pot at each treatment; the third group of pots received two additions of 2 per cent ground filter paper and the same amount of inorganic salts as the second group.   The moisture content of half of the cultures in each series was brought to optimum (about 20 per cent moisture), while the sand in the other half of the pots was saturated with water, allowing a layer of free water about 2 cm. deep to cover the surface of the sand. The pots were inoculated with a dilute suspension of a rich garden soil, covered with glass plates and incubated at 25 to 28°, water being added at frequent intervals to bring to original weight.   The cultures were analyzed 60 days after the second addition of organic matter.

The contents of the pots were well mixed, and aliquot portions of the moist mixture (including portions of the excess liquid where an excess of water was added) taken out into a series of flasks or beakers.   The residual organic matter in one portion of the sand mixture can be readily removed from the sand by constant washing upon a filter paper, the dry weight of which has previously been determined.   At first normal KCl is used for washing, so as to remove all the residual ammonia; after about 250 cc. of the filtrate has been collected from about 100 gm. of sand culture, distilled water is used for further washing. Ammonia is determined in the filtrate by adding some MgO and distilling into standard acid.   The sand is well stirred with consecutive portions of water until all traces of organic matter are removed.   Some of the sand is of course also transferred thereby upon the filter.   The paper, containing the organic matter with the adhering sand is dried at 80 to 90°C. to constant weight. The contents of the paper are then carefully transferred to an Erlenmeyer flask. An equal amount of 5 per cent NaOH solution is then added and the flasks are autoclaved, at 15 pounds pressure, for 30 minutes.   The contents of the flask, while still hot, are diluted with an equal quantity of distilled water and transferred carefully upon weighed ashless filter papers.   Every particle of sand in the flask is washed upon the filter; the residue is washed several times with water, dried to constant weight, at 80 to 90°C., and ignited.   The ash subtracted from the weight of the original residue gives the amount of organic matter left in the sand, free from ash and water-soluble constituents; the weight of the ash subtracted from the second residue gives the amount of

organic matter not soluble in 5 per cent NaOH solution. The NaOH solution and the washings are treated with an excess of 1:1 HCl. The precipitate is filtered, washed, dried, weighed, and ashed. The ash-free precipitate is recorded as "humus" (this preparation is the α-fraction of soil "humus," which is practically ash-free).

This method has one distinct disadvantage, namely, in the process of removing the organic matter from the sand by washing, all the water-soluble substances, including a large part of the bacterial cells, are washed out. By determining the total carbon and the total nitrogen content of the combined filtrate, however, the organic matter thus removed is measured. The "humus" was also extracted directly from aliquot portions of the sand culture (contain-

TABLE 1

Formation of "humus" from straw and cellulose in sand media, under aerobic and anaerobic conditions*

On the basis of 100 gm. of dry residue

TREATMENT			TOTAL NITROGEN	TOTAL AMMONIA NITROGEN	RESIDUAL ORGANIC MATTER FREE FROM ASH AND THE WATER-SOLUBLE PORTION	RESIDUAL CELLU-LOSE	ORGANIC MATTER EX-TRACTED BY NaOH (5 PER CENT SOLUTION)	"HUMUS" α-FRAC-TION FREE FROM ASH	
Nature of organic matter (4 per cent used)	Mois-ture	Nitro-gen and minerals						Total weight	Nitro-gen content
	per cent		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
Straw............	60	0	37.8	Trace	2,237	346	1,436	474	2.06
	100	0	36.8	Trace	2,918	1,065	1,603	636	1.84
	60	+	111.4	49.2	2,263	258	1,513	520	1.98
	100	+	110.6	53.2	2,705	376	1,647	621	1.70
Cellulose........	60	+	92.0	34.8	1,720	609	921	106	4.95
	100	+	86.6	47.7	3,618	3,208	111	30	....

* The ash-free, dry organic matter added in the case of straw was 3300 mgm. and in the case of cellulose 3700 mgm.

ing the organic matter) by treating with 5 per cent NaOH solution, then filtering and precipitating the "humus" (α-fraction) as usual. Cellulose and total nitrogen were determined in aliquot portions. Separate portions were used for moisture determinations and the results were all calculated on the basis of dry weight of the sand culture.

The data recorded in table 1 give an interesting insight into what is taking place when organic matter is decomposed in the soil. Out of 3300 mgm. of organic matter—free from moisture, ash, and water-soluble constituents—added to 100 gm. of sand in the form of straw, about 2250 mgm. was left under aerobic conditions, and about 2800 mgm. under anaerobic conditions, showing a much more rapid decomposition in the presence of sufficient aeration. The greater decomposition of the organic matter under aerobic conditions is largely

accounted for by the amount of cellulose decomposed. It was pointed out elsewhere (4) that bacteria capable of decomposing cellulose under anaerobic conditions are lacking in normal soils. When cellulose is introduced into a normal soil and sufficient water is added to make conditions anaerobic, no cellulose decomposition will take place before 2 or 3 months have elapsed, because of either a gradual development of the proper organisms or a gradual adaptation of a proper population already existing in the soil. Once such a population has become established, however, the celluloses will be rapidly decomposed. The excess of cellulose (determined directly) in the sand culture kept under anaerobic conditions over the aerobic soils accounts quantitatively for the smaller amount of organic matter decomposed.

By comparing the figures for residual organic matter, for residual cellulose, and for the organic matter which is soluble in 5 per cent sodium hydroxide, one can readily see that a large part of material has been synthesized in the process of decomposition of organic matter added to the sand medium, especially under aerobic conditions. In the case of straw kept at an optimum moisture content, out of the 2250 mgm. of organic matter (free from ash and water-soluble constituents) left, about 1520 mgm. is soluble in sodium hydroxide solution, and only 730 mgm. is insoluble. The insoluble portion consists of about 300 mgm. of cellulose, as determined quantitatively, and 430 mgm. of organic matter unaccounted for, including probably some lignins, fats, waxes, and largely pentosans. The original straw (free from ash and water-soluble materials) contained only about 30 per cent of material extracted by sodium hydroxide. This has increased, as a result of decomposition processes, to 67.5 per cent. This increase is due both to the relative increase of the lignins, which have not been decomposed to any extent, and to the substances synthesized by the microörganisms, which have utilized the celluloses as sources of energy. A large part of this protoplasm is soluble in sodium hydroxide and is precipitated by hydrochloric acid. The "humus" formed from the decomposition of straw in sand is thus due both to the substances of plant origin and to the synthesizing action of microörganisms.

This synthesizing action is brought out especially in the data obtained from the decomposition of pure cellulose in the sand cultures. Originally the sand and paper did not contain any substances soluble in dilute alkalies. The rapid decomposition of the cellulose under aerobic conditions resulted in a reduction of the cellulose from 3700 mgm. (pure, water-free cellulose) to 609 mgm. Of the 3091 mgm. of cellulose that has become decomposed, there is formed in the soil 1011 (1720 − 609) mgm. of organic matter, over 90 per cent of which (921 mgm.) is soluble in sodium hydroxide. That this newly formed organic matter is synthesized microbial protoplasm can be confirmed by the nitrogen data; the pots, in which cellulose has decomposed under aerobic conditions, contain 92.0 mgm. of total nitrogen (in 100 gm. of dry sand medium) and 34.8 mgm. of ammonia nitrogen, or 57 mgm. of nitrogen in the form of synthesized organic matter. This amounts to about 5.6 per cent nitrogen for

the synthesized organic matter in the soil. It is important to remember, in this connection, that fungus mycelium contains about 4 to 8 per cent nitrogen, with an average of about 5 to 6 per cent, as shown by Heukelekian and Waksman (1) in the study of decomposition of cellulose by pure cultures of fungi. In other words, *for 3091 mgm. of nitrogen-free cellulose decomposed, there has been synthesized 1011 mgm., or about one-third, of microbial protoplasm which contains 5.6 per cent nitrogen, and 90 per cent of which is soluble in sodium hydroxide.* These results show definitely that sodium hydroxide extracts from normal soils a complex mixture of organic materials or "humus" which consists largely of a nitrogen-poor lignin, introduced with the original organic matter added to the soil and which is resistant to decomposition, and a nitrogen rich constituent of microbial protoplasm synthesized in the soil.

This "humus" formation is illustrated in the data on the α-fraction of the "humus." It must be kept in mind that this is the part of the organic matter which is dissolved by the sodium hydroxide and precipitated by an excess of hydrochloric acid, in other words, that part of the soil organic matter which has most commonly been referred to as "humic acid." When washed with sufficient dilute hydrochloric acid and water, this fraction is practically free from ash. It has been pointed out elsewhere that, when obtained from normal cultivated soil, the nitrogen content of this α-fraction or "humic acid" is about 2.5 to 3.5 per cent. The amount of "humic acid" obtained from the sand medium, in which straw has been decomposing under aerobic conditions, is somewhat less than under anaerobic conditions, due to the fact that, with sufficient aeration, certain bacteria and actinomyces are capable of breaking down the lignin very slowly. Under anaerobic conditions, these organisms are not very active and the lignin passes largely into soil "humus." The nitrogen part of the "humus" is largely a result of the synthesizing activities of the microörganisms of the soil, as shown by the data on the "humus" formed from cellulose: the decomposition of about 3 gm. of cellulose, in the form of filter paper, resulted in the synthesis of about 1 gm. of organic matter, derived from the microbial cells, over 10 per cent of which gives all the characteristics of "humic acid," or the α-fraction of "humus." This synthesized "humic acid" contained 4.7 per cent nitrogen. The "humic acid" found in the pots receiving straw has been derived largely from the lignin of the straw and the synthesized microbial cells; the latter have contributed the nitrogen-bearing fraction; the total nitrogen content in the "humus" is considerably less under anaerobic conditions where less protoplasm has been synthesized. This is because, under aerobic conditions, fungi and aerobic bacteria are largely responsible for the decomposition of celluloses and under anaerobic conditions bacteria carry on this process; since the former synthesize more protoplasm than the latter, the "humic acid" or the α-fraction of "humus" found under aerobic conditions will contain more nitrogen, especially before that "humus" begins to decompose, in its turn.

Under anaerobic conditions, where fungi and aerobic bacteria are less active or are inactive, decomposition of the added organic matter proceeded much

more slowly, especially in the case of the celluloses. Although the total amount of organic matter decomposed under anaerobic conditions is considerably less than under aerobic conditions, more of the "humus," or of that part of alkali extractable material which is precipitated with an excess of hydrochloric acid, is left under anaerobic conditions. This is due largely to the fact that although the lignin may be somewhat decomposed under aerobic conditions, by the action of certain organisms—this question will be discussed in detail later—very little of it is decomposed under anaerobic conditions. A considerably smaller amount of "humus" is synthesized in the last case, as shown by the data for the decomposition of the ground filter paper.

Experiment 2. The formation of "humus" in the soil

The formation of "humus" in soil itself was studied in a manner similar to the studies in sand media, but the studies were continued for a considerable time. A rich sandy sassafrass soil was air dried, and 1000-gm. portions were placed in a series of glazed earthenware pots. Since the soil was acid, 2 gm. of $CaCO_3$ was added to each soil, thereby bringing the reaction to pH 6.2. A few of the pots with soil were left without organic matter, a few received an application of 1 per cent cellulose (in the form of filter paper) and 0.1 per cent $NaNO_3$, and a few received 1 per cent straw and 0.1 per cent $NaNO_3$. The soil moisture in half the pots was brought to 60 per cent saturation and in the other half fo full saturation, allowing an excess of water over the surface so as to make conditions anaerobic. Water was added at weekly intervals to keep the moisture at the same concentration. The pots were covered with glass plates and incubated at 25 to 28°C. After 5 months incubation, when most of the cellulose had been decomposed, 2 per cent filter paper and 0.2 per cent $NaNO_3$ were added to the cellulose pots and 2 per cent straw to the corresponding soils. The soils were again incubated for 10 weeks, and then analyzed. The cellulose determinations have shown that only about 0.1 per cent cellulose was left in the aerobic soils and 1.16 per cent in the anaerobic soils because of the abundant activities of the fungi in the former soil.

The α-fraction of the "humus" is that part of the soil organic matter which is soluble in alkalies (5 per cent NaOH) and is precipitated by an excess of acid. The β-fraction of the "humus" is that portion which is soluble both in an alkaline and in an acid solution and which is precipitated at a pH value of about 4.8, as shown before. (Table 2.)

The results seem to confirm quite definitely the observations made upon the formation of "humus" in the sand media. The soil itself contains a considerable amount of organic matter. It is interesting to note, in this connection, that the soil kept under aerobic conditions for $7\frac{1}{2}$ months had less organic matter in the "humus" fractions than the soils kept under anaerobic conditions. This is due entirely to the fact that "humus" is not decomposed to any extent when the soil is saturated with water, because the organisms capable of bring-

ing about this process are aerobic and are favored by a lower moisture content and by a neutral reaction—facts to be dwelt upon in a later contribution. This serves to throw interesting light upon the accumulation of organic matter ("humus") in soils kept saturated with water—as peat soils—as well as upon the rapid decomposition of organic matter in arid soils. The decomposition of the "humus" under aerobic conditions can be readily measured also by the formation of carbon dioxide and by the accumulation of nitrate nitrogen. Both of these phenomena run parallel because of the nearly constant ratio between the carbon and nitrogen in the soil. Since these soils were kept in closed containers, the nitrates accumulated and the excess of nitrate and ammonia nitrogen in the aerobic soil over the anaerobic soil more than balanced the lower nitrogen content in the two "humus" fractions in the aerobic soils. Under natural conditions, this soluble nitrogen would either be assimilated by

TABLE 2

Formation of "humus" in the soil from cellulose and straw, under aerobic and anaerobic conditions
On the basis of 100 gm. of dry soil

NATURE OF ORGANIC MATTER (3 PER CENT)	MOISTURE	"HUMUS," α-FRACTION, ASH FREE		"HUMUS," β-FRACTION, ASH FREE		TOTAL "HUMUS"	TOTAL NITROGEN IN"HUMUS"
		Total in 100 gm. of soil	Nitrogen content	Total in 100 gm. of soil	Nitrogen content		
	per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Control............ {	60	533	13.0	1,110	10.0	1,643	23.0
	100+	470	12.5	1,555	16.5	2,025	29.0
Cellulose........... {	60	668	18.5	1,253	11.0	1,921	29.5
	100+	575	18.3	1,518	17.8	2,093	36.1
Straw............... {	60	920	18.3	1,635	14.6	2,555	32.9
	100+	1,028	19.3	1,818	23.0	2,846	42.3

higher plants—wild or cultivated—or would be leached out. One of the reasons for the accumulation of "humus" under anaerobic conditions is the insufficient decomposition of the substances of plant (lignins) and microbiological origin.

The soils that have received an application of 3 per cent cellulose and sufficient nitrate nitrogen for the complete decomposition of the former, show a gain in "humus," especially under aerobic conditions. Cellulose is completely decomposed by microörganisms (1) under aerobic conditions, leaving only synthesized microbial protoplasm and one waste product—carbon dioxide. It is this synthesized protoplasm which contributes to the soil "humus," as demonstrated in the previous experiment and brought out also in table 2. The fact that the gain in the "humus," especially in the α-fraction, is directly of fungus origin can be illustrated by the increase in the nitrogen content in

this fraction—a gain of 135 mgm. in the α-fraction of 100 gm. of soil was accompanied by a gain of 5.5 mgm. of nitrogen in that fraction, or a little more than 4 per cent nitrogen. Where the gain in "humus" is due only to an increase in lignin content, there is a tendency to a diminution of the nitrogen content, since lignin is free from nitrogen; where the gain in "humus" is due both to lignin and to constituents of the microbial protoplasm, as when natural plant materials are added to the soil, there is a balance in the nitrogen content of the "humus," especially of the α-fraction. Under anaerobic conditions, cellulose is decomposed entirely by bacteria which synthesize only a very small amount of protoplasm under these conditions. The total gain in the amount of "humus" is very small, although an appreciable gain is found in the α-fraction, accompanied by a gain in the nitrogen content of this fraction.

The greatest gain in "humus" is found in the soils receiving 3 per cent of straw, especially under anaerobic conditions. A number of phenomena speak here for the contribution of the lignin to the soil "humus":

1. The increase in the "humic acid" (α-fraction) under anaerobic conditions is 558 mgm. $(1028 - 470)$; this is equivalent quantitatively to the amount of lignin added, the straw containing 18.8 per cent lignin ($188 \times 3 = 564$). It should be noted that the lignin comes down in the "humic acid" fraction and is not decomposed under anaerobic conditions; under aerobic conditions, there is a smaller increase in the "humic acid" because part of the lignin was no doubt decomposed.

2. Although the increase in the α-fraction of the "humus" ("humic acid") in the soils receiving the straw is much greater than in the soils receiving the pure cellulose (nearly three times as much under aerobic and over five times as much under anaerobic conditions), there is practically no gain in the nitrogen content. This is due to the fact that lignin theoretically contains no nitrogen; the gain in the nitrogen content of the "humus" comes from the ingredients of the microbial protoplasm contributing to the "humus" and to some extent of the protein of the straw. Since straw contains only about 70 to 75 per cent of the energy available for the activities of the soil microörganisms (monosaccharides, starches, celluloses, pentosans) that is offered by an equivalent amount of filter paper, a proportional amount of microbial protoplasm can be synthesized under the same conditions.

Straw contributes to the soil "humus," in addition to the lignins which are found in the α-fraction, a certain ingredient in the β-fraction, especially under aerobic conditions. Whether this is a constituent of the straw or a synthesized material which is absorbed by the aluminum of the β-fraction remains to be investigated.

The results of this experiment, as well as those of the previous one, point definitely to the fact that the α-fraction of the humus is formed in the soil by the lignin from the higher plants (and to a more limited extent by the fats and waxes) and by certain ingredients of the synthesized cells of microörganisms. These ingredients contribute largely to the nitrogen part of this "humus" fraction. The cells have been synthesized as a result of the utilization of the celluloses, pentosans, starches, monosaccharides, and other constituents of plant tissues, as sources of energy by the microörganisms, and the proteins or of available nitrogen compounds as sources of nitrogen.

Experiment 3.   Fungus mycelium as a source of humus in the soil

To learn more exactly the rôle of the cells of microörganisms as contributing agents to the soil "humus," the following experiment was carried out:

A quantity of mycelium of a green *Trichoderma*—a common cellulose decomposing organism in the soil—and of *A. niger*—a common mold in nature—was prepared by growing the organisms upon a synthetic (Czapek's) solution in large flasks until growth was at a maximum.   The mycelium was then filtered off through paper, washed with distilled water, and dried.   Two quantities of mycelium were thus obtained.   These were treated by the same procedure as was the straw described in the previous paper; namely, first they were extracted with ether, then with 95 per cent alcohol, then with cold water for 24 hours, and then with 5 per cent NaOH for 48 hours at room temperature,

TABLE 3

*Composition of fungus mycelium**

ORGANISM	TRICHODERMA	ASPERGILLUS
	per cent of total	*per cent of total*
Untreated mycelium.............................	(I)	(II)
Moisture content...............................	7.6	6.40
Ether extract.................................	4.2	.....
Alcohol extract...............................	8.1	8.00(III)
Cold water extract............................	17.3	15.10(IV)
5 per cent NaOH extract.......................	29.8	30.32(VI)
Loss on extraction with H_2SO_4...................	17.8	16.18
Insoluble residue..............................	15.2	24.00(VII)
NaOH extract precipitated with HCl..............	20.3	23.02(VIII)

* The Roman numerals designate the number of the preparation, obtained as a result of the particular treatment (as VI = preparation of *Aspergillus* mycelium, after NaOH extraction).

and finally they were treated with 2 per cent H_2SO_4 for 1 hour at boiling temperature.

Table 3 gives the analysis of the two fungus preparations.   Ether was found to extract 4.2 per cent of the fungus mycelium, alcohol 8.0 to 8.1 per cent, and cold water 15.1 to 17.3 per cent.   Five per cent sodium hydroxide extracted 29.8 per cent of the *Trichoderma* mycelium and 40.32 per cent of the *Aspergillus* mycelium, the larger part of which could again be precipitated by hydrochloric acid; in other words, the preparations of both fungi contained 20.3 and 23.02 per cent of material which comes down as the α-fraction of "humus" or as "humic acid."   The nitrogen contents of these fractions were 4.31 and 3.33 per cent respectively.   It is interesting to compare these data with those reported by Winterstein and Reuter (5) for higher fungi.   These investigators reported 4 per cent ether extractives (3.3 per cent fat and 0.5 per cent cholesterol), 12.0 per cent alcohol extractives (sugars, lecithin, bases, amino

bodies, purine bodies), and 28.0 per cent water-soluble substances (sugar, glycogen, purine bases, amino acids, etc.); the residue, comprising 46 per cent, was found to consist of 30 per cent protein, 10 per cent of an amorphous carbohydrate (para-iso-dextrin), and 6 per cent chitin.

Preparation VII, or the residue insoluble in dilute alkalies and dilute acids, probably consists largely of chitin. The presence of this preparation in the soil probably has something to do with the substance usually described as "humin" and "ulmin," or the most resistant part of the soil organic matter, as far as treatment with chemicals is concerned. It is comparable to cellulose, but it contains nitrogen and decomposes readily in the soil—to be sure more slowly than the soluble carbohydrates, starches and proteins, but it does not accumulate in the soil. It was not the purpose of this experiment to isolate any specific compounds from microbial protoplasm and to study their decomposition in the soil, but merely to separate the mycelium into several fractions with the idea of learning which of these decomposes more readily and whether any ingredients which may contribute to the soil "humus" are left in the soil.

Two-gram portions of the different preparations were added to a series of 100-gm. portions of washed sand or soil placed in long necked flasks. The sand cultures received also 20 mgm. of nitrogen in the form of dibasic ammonium phosphate and a trace of $MgSO_4$ and KCl. The sand cultures were sterilized and inoculated with a pure culture of *Trichoderma*, while the soil cultures were left unsterilized. The cultures were placed in the incubator (at 27 to 28°C.) and connected with the respiration apparatus; the CO_2 evolved was absorbed in standard barium hydroxide solution. After 34 days' incubation, the cultures were analyzed for residual ammonia, organic matter (in the sand cultures) and "humus" fraction in the soil.

The results indicate quite definitely that fungus mycelium, as represented by two of the most common filamentous fungi in the soil and in nature, consists of fractions which decompose with different degrees of rapidity. The removal of the ether-soluble fraction increases the degree of decomposition of the mycelium. The removal of the water-soluble fraction, however, tends to reduce the degree of decomposition of the mycelium; ether extracts substances which decompose with difficulty; while water removes the soluble sugars and amino acids which decompose readily. The most interesting portion is that removed by sodium hydroxide; it is largely nitrogenous in nature as indicated by the fact that whereas the mycelium of *Trichoderma* contained only 4.8 per cent nitrogen and the mycelium of *A. niger* 3.67 per cent nitrogen, the material extracted by sodium hydroxide contained 7.9 per cent nitrogen. The preparation obtained by precipitating this extract with hydrochloric acid contained 4.3 to 6.0 and 3.3 to 5.0 per cent nitrogen, respectively. The removal of this preparation does not prevent the decomposition of the residual material but reduces it somewhat in the sand culture and even increases it in the soil culture (fraction VI). The fraction left, after the alkali treatment, contains a considerable quantity of carbohydrate, amylose in nature, a large part of which

TABLE 4

The decomposition of fungus mycelium in sand by Trichoderma and in soil by mixed soil flora

NUMBER OF PREPARATION	MEDIUM	CARBON EVOLVED AS CO_2—INCUBATION						TOTAL C AS CO_2	C LIBERATED AS CO_2—CONTROL SUBTRACTED	AMMONIA N + NITRATE N CONTENT IN 100 GM. OF SAND OR SOIL	AMMONIA N FORMED FROM MYCELIUM	"HUMUS" (α AND β FRACTIONS) IN 100 GM. OF SOIL	
		2 days	5 days	10 days	15 days	21 days	34 days					Total	Control subtracted
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
I	Sand	24.5	44.4	45.1	73.7	36.6	7.9	234.2	224.2	47.2	27.2		
II	Sand	21.1	41.7	63.0	71.5	34.4	40.6	272.3	262.3	30.9	10.9		
III	Sand	30.7	30.2	73.0	70.1	48.2	56.3	308.5	298.5	35.4	15.4		
IV	Sand	....	53.4	102.2	68.0	48.4	8.1	280.1	270.1	33.5	13.5		
VI	Sand	....	....	32.9	65.3	45.9	62.3	206.4	196.4	11.9	−8.1		
VII	Sand	....	68.2	62.4	72.6	60.2	21.4	284.8	274.8	24.1	4.1		
VIII	Sand	....	....	....	43.2	21.3	34.6	99.1	89.1	19.4	−0.6		
Control	Sand	....	....	3.4	43.2	21.3	6.6	10.0	....	20.0	....		
I	Soil	52.9	55.4	97.0	61.9	61.4	50.6	379.2	335.1	18.2	15.2	1,010	461
II	Soil	24.3	52.3	22.0	74.0	45.2	48.9	266.7	222.6	41.6	38.6	797	248
III	Soil	18.6	37.9	73.6	65.7	63.7	38.1	297.6	253.5	20.4	17.4	933	384
IV	Soil	13.8	22.7	56.3	77.0	53.2	59.4	282.4	238.3	16.2	13.2	747	198
VI	Soil	21.9	53.4	78.5	70.5	46.9	56.7	327.9	283.8	16.0	13.0	823	274
VII	Soil	....	24.3	64.7	42.1	109.2	76.6	316.8	116.8	8.6	5.6	796	247
VIII	Soil	....	35.5	27.2	13.8	23.4	30.9	130.8	86.7	4.2	1.2	1,102	553
Control	Soil	....	8.5	....	16.8	13.8	5.0	44.1	....	3.0	....	549	....

is made soluble by treatment with 2 per cent sulfuric acid and which decomposes very readily. It is so readily decomposed by *Trichoderma* in sand culture that a considerable amount of nitrogen has to be obtained from the additional ammonium salt. In the soil, however, the decomposition of fraction VI is more rapid, and sufficient nitrogen is liberated from the other fractions by different organisms.

Portion VIII, or that part of the fungus mycelium which is dissolved by sodium hydroxide and precipitated by hydrochloric acid, gives all the characteristics of the α-fraction of soil "humus." Like lignin and the α-fraction itself obtained from soil, it decomposes only very slowly. When added to soil it greatly increases the "humus" content of the soil. (Table 4.) Since alka-

TABLE 5

Nitrogen balance of cell substance of Trichoderma

PREPARATION	AMOUNT TAKEN OR YIELD	NITROGEN CONTENT FOR 3 GM. OF ORIGINAL MATERIAL
	mgm.	*mgm.*
Dry mycelium of Trichoderma..............................	3,000	151.8
Cold NaOH extract:		
Hot HCl precipitate...................................	280	20.6
Solution left...	...	69.8
Hot NaOH extract (following the cold extraction):		
Hot HCl precipitate...................................	251	4.9
Solution left...	...	32.6
Residue, after NaOH extraction............................	987	14.4
Total nitrogen accounted for............................		142.3
Lignin content of mycelium (Schwalbe method)..............	654	8.7
Lignin content of NaOH extract precipitated with hot HCl....	531	25.5

lies extract also certain hemicelluloses, the part of preparation VIII which has undergone decomposition, is this non-nitrogenous ingredient, as indicated by the fact that practically no ammonia is liberated as a result of its decomposition. Whereas the chitin preparation (insoluble in water, alkali and dilute acid—VII) decomposed very readily, as indicated by the evolution of CO_2 with the liberation of small amounts of ammonia, the "humus" preparation, or that part of the fungus mycelium which is soluble in dilute alkalies and precipitated by acids, decomposed only very slowly. Only 89.1 mgm. of CO_2 was given off in the sand medium and 86.7 mgm. in the soil medium, 0.6 mgm. of ammonia nitrogen was consumed in the sand medium, and 1.2 mgm. of ammonia nitrogen was liberated in the soil. This small amount of CO_2 is a result of decomposition of the hemicelluloses which accompanied the "humus"

fraction extracted from the fungus mycelium. In the further studies on the decomposition of the microbial protoplasm, which will be reported later, it has been found that when the "humus" fraction is boiled after precipitation with hydrochloric acid, the decomposition of this fraction by microörganisms is reduced to a minimum. In other words, the alkaline extract of fungus mycelium precipitated with hydrochloric acid and boiled in the presence of an excess of acid, gives a preparation which is identical in its resistance to decomposition to soil organic matter and to lignins.

Table 5 gives a balance of the nitrogen of the cell substance of *Trichoderma* grown on a synthetic medium, showing that most of the nitrogen is extracted by sodium hydroxide and remains in solution, after the hot hydrochloric acid precipitate is removed. "Humus"-like preparations containing varying amounts of nitrogen are obtained from this mycelium.

The results presented in this paper thus indicate definitely that the activities of microörganisms in the soil result in the synthesis of substances which possess all the characteristics of soil "humus." These substances are constant ingredients of the cells of microörganisms and are of a higher nitrogen content than the soil "humus." Their exact chemical nature remains to be determined.

The results also indicate that when plant residues are added to the soil, most of their constituents, including the monosaccharides, celluloses, and pentosans, are decomposed with comparative rapidity, although some resist decomposition. The microörganisms decomposing the various plant constituents may synthesize microbial protoplasm equivalent in weight to 20 to 30 per cent of the plant materials decomposed. This protoplasm can be further decomposed, especially in normal soils; however, a part of it resists decomposition. This constituent or group of constituents of the microbial protoplasm, a part of which is soluble in alkalies and precipitated by hydrochloric acid, contributes definitely to the soil "humus" or soil organic matter, which resists decomposition and may persist in the soil for a considerable period of time. This synthesized microbial protoplasm is probably the important source of the nitrogen found in the soil "humus."

Soil "humus," or that part of the soil organic matter which is soluble in alkalies, is thus found to be made up of substances originating from two different sources: 1. Lignins and other constituents of plants and plant residues. 2. Certain ingredients of the protoplasm synthesized by soil microörganisms.

GENERAL SUMMARY

A careful search of the extensive literature on the nature of soil organic matter or "humus" reveals two tendencies: 1. To describe "humus" and the so-called "humic acids" as definite compounds, the composition of which can be readily ascertained by analysis, as in the work of Berzelius, Berthelot, Oden and others; 2. To consider "humus" in the light of colloidal complexes whereby all reactions, in which soil organic matter is involved, are ascribed to

adsorption phenomena, as in the work of van Bemmelen, Baumann, and others. In both instances, the dynamic condition of the soil organic matter was not taken into consideration, and "humus" was looked upon as a mass of dead debris, originating by some obscure processes. The recent investigations of Trusov, Fischer and others, have definitely established the fact, suggested by various workers previously, that the lignins of the various constituents of natural organic matter are most resistant to decomposition and contribute largely to the soil "humus." However, these contributions as well fail to account for all the properties of the soil organic matter, the chief of which is (a) the presence of a definite amount of nitrogen in the "humus," and (b) the fact that, under certain conditions, "humus" accumulates, as in peat bogs, whereas under other conditions, "humus" tends to decompose readily, as in well aerated and limed soils.

The terms "humus," "humic acid," and other designations given to various preparations of soil organic matter have no justification either biologically or chemically. Only in popular usage can the complex soil organic matter, in contradistinction to the natural organic matter, be spoken of as soil "humus," meaning those amorphous dark-brown substances which are characteristic of soils rich in organic matter. To speak of the degree of "humification" or change in color of the organic matter in the soil as a result of various unknown agencies is merely to use terms which do not stand for any well understood processes and will no more serve to advance our knowledge of the subject in question than putting a label upon an unknown phenomenon and believing that it has thus been solved.

A study of the nature of soil organic matter or "humus" involves a number of complex problems, dealing with (a) the origin of this organic matter, (b) its chemical nature and its rôle in the soil processes and (c) its decomposition in the soil. The preliminary experiments reported in these contributions tend to throw light upon the first two problems, and a further detailed study of these as well as of the third problem is now in progress and the results will be reported later. From these preliminary studies the following conclusions may be drawn:

1. By the use of dilute sodium hydroxide, the soil organic matter can be separated into four distinct groups: (a) the part insoluble in dilute alkali, even under pressure; (b) the part soluble in alkali and precipitaed by an excess of hot hydrochloric acid; (c) the part soluble in alkali and in acid, but precipitated at a definite isoelectric point, namely at pH 4.8 to 5.0, an organic-inorganic complex; (d) the part made soluble in water, as a result of treatment of the soil with the alkali. The third fraction may be absent altogether in peat soils, but it is present abundantly in mineral soils, consisting of about 50 to 70 per cent aluminum and other bases and 25 to 40 per cent organic matter. The presence and absence of this fraction allows a differentiation between peat and mineral soils. The second fraction, equivalent to the so-called "humic acids," is practically free from ash and contains from 2 to 4 per cent nitrogen.

2. When organic matter is added to the soil, the various sugars, starches, hemicelluloses, celluloses, and proteins are rapidly decomposed. The lignins and, to a lesser extent, the fat and waxes resist decomposition under anaerobic conditions, as in peat soils, and are slowly decomposed under aerobic conditions by certain groups of organisms.

3. Pure or mixed cultures of soil fungi and bacteria rapidly attack the various constituents of the natural organic materials added to the soil, converting a part of the carbon into microbial protoplasm. A definite amount of nitrogen is thereby assimilated and changed from an inorganic into an organic form or from plant proteins into microbial protoplasm. A part of this protoplasm gives all the reactions characteristic of "humus" and is rather rich in nitrogen.

4. The soil "humus" or that part of the soil organic matter which gives to it its dark color and which is more or less resistant to decomposition, is a result of the accumulation of substances of plant origin—on the one hand, largely the lignins and to some extent the fats and waxes and perhaps certain nitrogenous substances, and on the other hand, substances synthesized by microörganisms, nitrogenous in nature.[2]

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[2] The author is indebted to Mr. R. Dubos of this laboratory for assistance in carrying out the various cellulose determinations. The method used for determining the cellulose content of straw will be described in detail later.

THE RELATION OF MANGANESE AND IRON TO A LIME-INDUCED CHLOROSIS[1]

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INTRODUCTION

A chlorotic condition has been noted frequently with various crops—particularly oats, spinach, lettuce, corn, beets, and beans (4)—on soils limed to the neutral point on the experimental plats of this station. Such lime-induced chlorosis of field and garden crops, which is not a new thing, has usually been attributed to an iron deficiency. Applications of soluble iron compounds have usually been found to cure it (3), but repeated attempts to relate the condition found at this station to an iron deficiency, or to correct it by supplying iron in various ways, have failed completely. Very small applications of manganese compounds have alleviated the malady, and its occurrence is found to be associated with a very low manganese content of the affected plants as compared with that of normal plants on similar but more acid soils.

IRON AND MAGNESIUM INEFFECTIVE AS CORRECTIVES

The chlorotic crops from the heavily-limed plats have been analyzed repeatedly for iron, with conflicting results. Oats showed marked chlorosis on the heavily-limed plat of a series of varying acidity in 1917. Analyses were made of the good oats on the more acid plats and both of the good and of the chlorotic oats on the less acid plat, with the results given in table 1.

The iron content of the chlorotic plants was found to be less than that of the green plants on the same plat (29) but both of these had a much higher iron content than those from the more acid plat (23).

Similar chlorotic conditions were observed with beans growing on certain of the plats with different kinds of lime. Chlorosis was particularly severe on plat 74, which had been heavily limed with high-calcium hydroxide, and on plat 80, which had been equally heavily limed with high-magnesium hydroxide. The beans on the control plat (82) which had not been limed, showed no signs of chlorosis.

Samples of leaves from the chlorotic and non-chlorotic plants were gathered in duplicate by two different people and analyzed for calcium oxide (CaO), magnesium oxide (MgO), and iron content, with the results given in table 2.

[1] Contribution 330 of the Rhode Island Agricultural Experiment Station, Kingston.

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The green, healthy plants had a lower content of both iron and magnesium than the chlorotic plants; therefore the abnormal condition cannot be attributed in this case to a deficiency of either iron or magnesium (2).

In 1919, on the Phosphate Experiment plats, receiving different treatments as regards calcium oxide (CaO) and phosphoric acid (P_2O_5), similar chlorotic

TABLE 1

Analysis of spring oats, 1919

PLAT NUMBER	CONDITION OF OATS	Fe CONTENT ON DRY MATTER BASIS	CaO REQUIREMENT*
		p.p.m.	*pounds*
23	Good	227	5,482
25	Good	. . .	4,228
27	Good	907	4,452
29	Good and green	676	3,046
29	Chlorotic	475	3,046

* Upper 12 inches of soil by the Ammonia Method (5).

TABLE 2

Average calcium, magnesium, and iron content of chlorotic and non-chlorotic leaves of beans, 1917

PLAT NUMBER	TREATMENT	CONDITION OF		CaO in DRY MATTER	MgO in DRY MATTER	Fe IN DRY MATTER
		Leaves	Soil			
				per cent	*per cent*	*p.p.m.*
74	High-calcium hydroxide	Chlorotic	Neutral	4.9	1.0	59.3
80	High-magnesium hydroxide	Chlorotic	Neutral	4.3	2.1	51.9
82	No lime	Green	Acid	3.4	0.8	37.9

TABLE 3

Iron content of oats and wheat, 1919

PLAT NUMBER	TREATMENT		CONDITION OF PLANTS	Fe CONTENT ON DRY MATTER BASIS	
	P_2O_5	CaO		Oats	Wheat
				p.p.m.	*p.p.m.*
58	Ground bone	Little	Green	780	. . .
68	None	Little	Green	340	480
53	Acid phosphate	Much	. . . . . .	900	890
57	Ground bone	Much	Chlorotic	440	440

effects were observed on oats and wheat, and sample plants were again analyzed (table 3).

The iron content of the chlorotic plants on the highly-limed bone plat was low, but not so low in the case of oats and not materially lower in the case of of wheat than of the green plants on the acid plat (68).

In the spring of 1922, spinach was found to be chlorotic on the heavily-

limed, north end of plat 128, and healthy on the lesser-limed, south end. The iron content of the spinach from 128N was 1232 p.p.m., and the pH was 7.0; and the iron content of the spinach from 128S, 815 p.p.m. with pH 6.0. The lesser-limed, healthy spinach had a lower iron content than the chlorotic spinach from the heavily-limed plat, the reaction of which was neutral.

In the spring of 1921, the plats given in table 3 were again in oats and comparisons were made of the acidities of the plats on which the plants were chlorotic or healthy. (Table 4.)

Here the relation to acidity is clear; the plats that were neutral being chlorotic, and the acid plats healthy. The oats on the neutral soils yielded less than a half crop.

Similar chlorotic symptoms had been noted previously on highly-limed plats, with corn and other crops. In 1922, these Phosphate Experiment plats were planted to carrots, corn, mangels, oats, onions, and potatoes. Of these only

TABLE 4

Soil acidity and condition of oats, May, 1921

| PLAT NUMBER | TREATMENT | | CONDITION OF PLANTS | SOIL REACTION |
	P_2O_5	CaO		
				pH
57	Ground bone	High	Badly chlorotic	7.5
58	Ground bone	Low	Green	6.8
59N*	Thomas slag	High	Chlorotic	7.7
59S†	Thomas slag	Less	Green	6.8
60N*	Thomas slag	Little	Green	6.0
60S†	Thomas slag	None	Green	5.4

* North half.
† South half.

the mangels and onions yielded more on the higher-limed than on the lesser-limed areas. Most of the other crops were chlorotic on the limed areas. This was especially true of corn, and injections with a variety of iron compounds in solution were made with negative results on the chlorotic corn plants.

Chlorosis of the crops on the heavily-limed plats continued to be noted in 1923 and 1924, and in 1924 this was even observed in a slightly acid soil which had previously been limed to neutrality. Thus the previous treatment appeared to have so affected the soil as to continue the chlorotic condition of the crops even for a time after the soil had become acid.

The previous experiments having yielded only negative results in an attempt to cure this lime-induced chlorosis, tests were planned for 1925 to ascertain whether ferrous iron or reducing agents which might convert ferric into ferrous iron would prove beneficial. The spinach on the market garden area having become chlorotic, on May 5, when the plants were a month old, the following

treatments (8) were tried on the odd-numbered rows of plat 101, the even ones having been left as controls:

Spraying with tap water; sulfuric acid $0.0001M$; mono-potassium phosphate 2 per cent; ammonium nitrate 0.08 per cent; potassium sulfate 1 per cent; ammonium citrate $0.001M$; acidulated with citric acid; ferric ammonium citrate (0.02 per cent ferric citrate and ammonium hydroxide to pH 6.5); ferrous sulfate 0.02 per cent; manganous sulfate 0.004 per cent; formaldehyde 0.04 per cent, and iron powder sprinkled along the row. (Unless otherwise specified in this paper reference is made to c. p. crystalline salts.) Thus each of the principal fertilizer ingredients was tried; also iron as citrate and as ferrous sulfate. Manganous sulfate ($MnSO_4 \cdot 4H_2O$) and formalin were used because they are reducing agents and might tend to convert ferric iron already present in the soil to the ferrous state.

In some instances the spraying of ferrous sulfate on the leaves, as of pineapples (6), subjected to too much manganese, has been found effective in curing an iron deficiency where applications to the soil have proved ineffective. Accordingly, the leaves of a few plants were injected with ferric ammonium citrate $0.001M$, and ferrous sulfate $0.004M$ (about 1 per cent solution). The citrate killed the leaves, and the ferrous sulfate produced no visible effect, the leaves remaining chlorotic. The spray applications likewise gave negative results, except in the case of the manganous sulfate. The plants sprayed with manganous sulfate regained a normal green color and resumed active growth 4 days after treatment, whereas the remainder of the spinach on the plat was chlorotic.

Applications of commercial iron sulfate at the rate of about 200 pounds per acre were also made to areas of chlorotic spinach and oats on plats which had been limed to the neutral point. These likewise produced no visible effect.

The applications of iron to the soil, sprayed on the plants, and injected into the plants, have given only negative results with the lime-induced chlorosis experienced at this station. Further, the percentage iron content of the healthy plants on acid soils is not generally greater, and is frequently less, than that of the chlorotic plants. This lime-induced chlorosis seems not to be due to an iron deficiency The chlorotic symptoms appear whether the soil is limed with high-magnesium or high-calcium lime, so the difficulty cannot be ascribed to a magnesium deficiency (2).

MANGANESE AS A CURE

Manganous sulfate was found to cure very promptly the chlorosis in spinach (7), in oats, in beets, and in beans on the nearly-neutral soils. Later, manganous chloride and potassium permanganate were used with the same sort of benefit. Extremely small amounts of manganese applied as sprays to the plants and soil, were found to be effective.

ANALYTICAL METHODS

Chemical determinations of the manganese content were made on both tissue and plant solution. Fresh plants were thoroughly macerated in a Nixtamel mill and the solution was filtered through fine-mesh silk. The solution was then decolorized by shaking with carbon black and heating to coagulation. After filtering and making up the solution to volume, aliquots were freed from organic matter by the wet combustion method using concentrated sulfuric and nitric acids. Then the color was developed by the use of the periodate method of oxidation (9) and the comparisons were made against standard potassium permanganate in a Kennicott-Sargent colorimeter. For the determinations of total manganese in plant tissue, the roots and tops of the plants were separated, carefully washed free from adhering soil particles, dried, and powdered. A 4- to 5-gm. sample was ashed in a platinum dish and the ash fused with 10 to 15 gm. of potassium bisulfate. After cooling, the dish and contents were immersed in dilute sulfuric acid and the whole heated to boiling to insure complete disintegration of the fused mass. The mixture was cooled and filtered through an asbestos Gooch crucible, the filter washed free from acid, and the filtrate transferred to a beaker for oxidation. The oxidation and colorimetric determinations were carried out as outlined above with the plant solution.[2]

The determinations of soil pH were made electrometrically and the "lime requirement" was determined according to the modified Jones' calcium-acetate method (1).

EXPERIMENTAL DATA

Strong solutions of iron salts injected into the tissues killed portions of the leaves, weaker solutions having no visible effect. Injections of manganous sulfate solutions into the tissues were beneficial.

On May 11, 1925, 6 days after treatment, the plants which had been sprayed with the manganous sulfate had become darker green and had started active growth. Those given the other treatments were more chlorotic than before, and were indistinguishable in appearance from the remainder of the plat.

On May 11, manganous sulfate solution was sprayed at the rate of 0.3 pound per acre and 0.15 pound per acre on two separate areas of approximately 120 square feet of plat 101.

On May 15, 4 days after these applications, the area treated with 0.3 pound per acre was already green and recovering from chlorosis, whereas the areas treated with 0.15 pound per acre showed no apparent benefit.

On May 14, applications of ferrous sulfate $0.001M$, manganous sulfate $0.0002M$, and potassium permanganate $0.0002M$ were made at the rate of 17 liters per 15 feet of row (in the case of manganous sulfate this amounted to

[2] The authors are indebted to J. S. McHargue for the suggestion of the method for the determination of total manganese.

about 1 pound of manganese per acre) on chlorotic spinach growing on the heavily-limed, neutral soil of plat 74S. Four days later, the plants treated with both the potassium permanganate and the manganous sulfate were observed to be regaining a good deep green color. The benefit was more evident with the manganous sulfate than with the potassium permanganate.

On May 23, a portion of the chlorotic spinach on each of the plats, 74, 76, 78 and 80, which were nearly neutral, and on 82 which was similarly fertilized but unlimed and strongly acid, was sprayed with manganous sulfate at the rate of about 0.5 pound per acre of manganese. This treatment was repeated on May 28, except on the acid plat 82, on which the spinach was already nearly dead because of aluminum toxicity and which at no time had shown any symptoms of the typical lime-induced chlorosis.

On June 3, each of these treated areas on the nearly-neutral plats showed uniformly green and good spinach, far superior to that on the untreated areas, which was very yellow, with large dead spots in the leaves.

TABLE 5

Comparative yields of spring spinach with and without manganese treatment, 1925

PLAT NUMBER	TREATMENT	NORTHWEST QUARTER, 1 POUND Mn PER ACRE AS MnSO₄·4H₂O	SOUTHEAST QUARTER, 1 POUND Mn PER ACRE AS KMnO₄	NORTHEAST QUARTER, NO TREATMENT	SOUTHWEST QUARTER, NO TREATMENT
74	High-calcium hydroxide, extra lime	42.5	25.5	15.5	24.5
76	High-magnesium limestone	56.7	63.5	46.5	59.0
78	High-calcium limestone	67.0	34.0	36.5	36.0
80	High-magnesium hydroxide	56.5	37.5	19.0	29.5
82	No lime	0	0	0	0
Average increase due to treatment, *per cent*............................		67	20		

On June 11, this area of spinach was harvested. The yields in pounds, green weight, are given in table 5.

The manganous sulfate was much more effective than the potassium permanganate in curing the chlorotic condition. Not only was the yield of spinach increased 67 per cent by the manganous sulfate treatment, but the spinach from the treated area was bright green and of good quality, whereas that from the untreated areas was so poor that it was unsalable.

The manganese content of the plant solution of the untreated plants was less than ten-millionths per cent and was not measurable. The manganese content of the whole plants at harvest was very small, but reflected quite clearly the treatment given. (Table 6.)

The manganese content of the plants was increased by the addition of manganese to the soil. The plants on somewhat acid soils (plats 29 and 53) had a

higher manganese content than those which were chlorotic (plat 76), although none of these three lots had been supplied with extra manganese.

The results with the manganese treatment of chlorotic spinach on nearly-neutral soils having been uniformly successful, a similar treatment with manganous chloride was employed in the fall on large areas to obtain more yield data. On September 18, 22 days after planting, 8 pounds of manganous chlo-

TABLE 6

Manganese content and condition of crop of fall spinach

PLAT NUMBER	TREATMENT		CONDITION OF CROP	YIELD PER ACRE	Mn IN DRY MATTER	Mn REMOVED PER ACRE	SOIL REACTION
	Fertilizer	1 pound Mn as					
				tons	*p.p.m.*	*pounds*	*pH*
76	High-magnesium limestone	None	Chlorotic	9.92	23.3	0.037	7.0
76	High-magnesium limestone	$MnSO_4 \cdot 4H_2O$	Good	12.10	34.2	0.067	7.0
76	High-magnesium limestone	$KMnO_4$	Good	13.91	60.0	0.125	7.0
29	Chemicals	None	Good	9.73	53.6	0.100	5.7
53M.G.*	Chemicals and manure	None	Fair	6.26	54.8	0.069	7.3

* M.G. = market garden plat.

TABLE 7

Fertilization of fall spinach on market garden plats

PLAT NUMBER	TREATMENT	
	Manure	Chemicals
	tons	
74	32	None
85*	32	None
86	16	Standard + extra K
87	..	Standard + peat + extra chemicals
115	16	Standard
116	16	Standard + extra N
117	16	Standard + extra P
196†	16	Standard

* Soil reaction, pH 7.6.
† Soil reaction, pH 6.8.

ride per acre, or approximately $2\frac{1}{4}$ pounds of manganese, was applied to one-half of each of 9 plats of spinach, the other half plat remaining untreated. The plats and their fertilizer treatments are given in table 7.

On September 25, the untreated spinach on all plats except 196 was becoming very yellow, and there were brown, dead spots in the leaves. All of the areas sprayed with manganese had quite healthy dark green leaves, as had all of the

spinach on plat 196, both the treated and untreated areas being apparently alike in appearance and vigor. The spinach on these plats was harvested on October 20 to 29. Table 8 shows the yields in bushels per acre.

The yield was significantly increased by the manganese treatment on all plats except 196, on which the soil was more acid. The chlorosis difficulty seems to be associated entirely with neutral or alkaline soils. It has not appeared with any crops on soils with a pH of 6.8 or less.

TABLE 8

Comparative yields of fall spinach with and without manganese treatment on the market garden plats, 1925

PLAT NUMBER	UNTREATED HALF	TREATED HALF*	INCREASE DUE TO Mn
	bushels†	*bushels*	*per cent*
74	143	450	215
85	415	725	75
86	1,080	1,235	14
87	615	950	54
115	930	1,215	31
116	1,075	1,345	25
117	1,090	1,310	20
196	1,170	1,170	0

* 8 pounds Mn per acre.
† One bushel of spinach equals 12 pounds green weight.

TABLE 9

Comparative manganese content of spinach with and without treatment on the market garden plats, 1925

PLAT NUMBER	ROTATION	TREATMENT Mn PER ACRE	H_2O	Mn IN DRY MATTER	Mn IN PLANTS PER ACRE	SOIL REACTION	CaO REQUIRE-MENT
		pounds	*per cent*	*p.p.m.*	*pounds*	*pH*	
115	W	None	93.11	51.8	0.040	...	...
115	W	2	89.05	92.4	0.148	...	...
85	W	None	85.84	51.8	0.037	7.6	380
85	W	2	87.09	103.0	0.116	7.6	...
196	W	None	85.01	56.1	0.098	6.8	810
196	W	2	83.74	57.5	0.121	6.8	...

The manganese content of the harvested spinach plants from some of these plats is given in table 9.

On plat 196, where the soil was very acid, the addition of manganese at the rate of 8 pounds per acre neither improved the yield nor greatly increased the manganese content of the plants. On plats 115 and 85 the appearance of the plants and the yields of spinach were substantially improved by the manganese treatment; and the manganese content of the plants was also nearly doubled in each case. The conclusion seems reasonable that spinach requires manganese

(7), and that on these highly-limed soils there is a deficiency of available manganese which is overcome by the surface application of 8 pounds per acre in this case. That the amount required is very small is evident from the small amounts found in the healthy plants (60 p.p.m.) and the small amount of this element removed by the crop (0.1 pound per acre).

OATS

In preliminary tests, oats which were chlorotic on heavily-limed soils, were benefited as was the spinach. Accordingly on May 28, when the oat cover crops were 54 days old and beginning to appear chlorotic, one-fourth of each of the market garden plats 77, 107, 91, 92, 121, and 122 was sprayed with manganous sulfate at the rate of 4 pounds of salt (1 pound of Mn) per acre. On June 3, six days after this treatment, the treated one-fourth of each plat was distinctly improved, having recovered a bright-green healthy color. The untreated areas showed symptoms similar to those of the spinach; the leaves became yellow, brown dead spots appeared in them, and growth was stunted.

TABLE 10

Comparative manganese content of oats with and without treatment on the market garden plats, 1925

PLAT NUMBER	ROTATION	TREATMENT Mn PER ACRE	H₂O	Mn ON DRY MATTER BASIS	pH OF SOIL	CaO REQUIREMENT
		pounds	*per cent*	*p.p.m.*		
107	X	None	88.9	25.0	. . .	. . .
107	X	1	89.4	27.1	. . .	. . .
92	X	1	90.0	40.1	7.3	473

Samples of a definite area were cut from treated and untreated sections of each plat, and the green weights determined. The weight of the forage on the manganese-treated area was 67 per cent greater than on the untreated areas.

The analyses of samples of oats for manganese content are given in table 10.

With oats as with spinach, the addition of very small amounts of manganese restored the health of the plants and also increased the yields and manganese content.

The above experiments with spinach and oats clearly indicate that the lime-induced chlorosis found with them was cured by applications of manganese salts—either manganous salts or permanganate. Further, the chlorotic plants had a lower manganese content than healthy plants on more acid soils.

The apparent manganese requirement of the plants is extremely small. Expressed in per cent of the dry matter, it was between 0.002 and 0.006 per cent for oats and spinach.

GENERAL SUMMARY

1. Chlorosis in this case is not due to a deficiency of iron, as the iron content of the chlorotic plants equalled or exceeded that of normal plants, and iron treatment was ineffective in curing the malady.

2. Chlorosis appeared on soils treated with both high-magnesium and high-calcium lime, thus indicating that a magnesium deficiency was not causing the trouble.

3. Small amounts of manganese salts cured the chlorotic condition.

4. The chlorotic plants had a lower manganese content than normal plants.

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A CONTRIBUTION TO THE STUDY OF INTERRELATIONS BE- TWEEN THE TEMPERATURE OF THE SOIL AND OF THE ATMOSPHERE AND A NEW TYPE OF THERMOMETER FOR SUCH STUDY[1]

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The actual amount of heat reaching the surface of the earth is only that portion of the sun's rays that passes unabsorbed through the atmosphere. The moisture content of the soil has a great influence in determining the temperature of the soil because of the higher specific heat of wet soil, which is close to 1 whereas that of dry soil is approximately 0.2. The surface layer of the soil frequently is hotter than the air, especially on sunny days in hot climates. Leather (8) states that the maximum temperature at Pusa (India) is 20°C. above the maximum air temperature in the shade.

Russell (12) summarizes the factors influencing soil temperatures as follows:

(*a*) A south slope is warmer than a north slope.

(*b*) Bare land is warmer than land covered with vegetation, excepting during winter months.

(*c*) Soil exposed to the sun's rays is often hotter than the air, and is subject to considerable temperature variations, which, however, only slowly affect layers three or more inches deep.

(*d*) Moist soil, being a better conductor than dry soil, is much more uniform in temperature.

(*e*) The top 6 inches of soil has a higher mean temperature than the air both in summer and in winter. At 6 inches the warmer part of the day centers around 5.30 p.m., and the cooler part around 9.30 a.m.

(*f*) The warming of the soil in spring is facilitated by drying; the cooling in autumn is increased by clear nights and diminished by rain [Keen and Russell, (7)].

The temperature of the soil and of the atmosphere is determined by certain laws and the importance of temperature as one of the climatic elements is well illustrated in the "Climatic Laws" formulated by Visher (14) in which he gives eleven meteorological laws which relate to climate and which, he shows, refer to a cycle due to their joint action. He states

The sun rises; its rays penetrate the air but are absorbed by the dark soil which thereby is warmed and gives out long heat rays which are absorbed by the air. As the air becomes

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warm it expands, winds are set up, and the heated air is moved away and warms other places, where the sun's heating is less effective.   Sometimes the air rises and is therefore cooled adiabatically, occasionally enough so that its moisture is partly condensed with a liberation of heat which still further warms the air.   Gradually the heat escapes outward into space or downward to cooler earth and water until the air is no longer relatively warm.   Then gravity pulls it down to the earth's surface where it awaits another warming the following day.

The temperature of the air and of the soil is to a great extent beyond the control of man.   The latter, however, by certain soil management methods can be slightly altered.   This is climatically perhaps unimportant but practically is worthy of careful consideration (9).

In the cranberry marshes of Wisconsin it was noted by Whitson (15) in 1905 that patches of frost occurred on level land where the marshes were poorly drained or covered with weeds, grass, or moss; whereas clean, well-drained, or sanded lands often escaped.   That cultivation, draining, and sanding are means of frost control in the marshes of Wisconsin was also noted by Cox (3) who further stated that because of radiation from the warm soil it is practically impossible for frost to occur in the bogs on the first cool night following a warm spell but that it is likely to occur on the second night after the soil has become cold.   The lowest temperature in the marshes is usually where the vegetation is heavy and the soil poorly drained, because the soil cannot heat up during the day.   When a soil is bared it becomes heated by the sun's rays according to the nature and the color of its particles and according to its humidity.   Seeley (13) emphasized the fact that the temperature of a soil covered with vegetation is dependent more upon the height of the plants and their thickness over the surface of the ground than upon the kind of a plant.   In a forest the loss of heat by radiation at night is checked by the forest canopy.   In a comparison between parks and timbered areas, Pearson (11) found that the mean annual temperature in the forest was 2.7°F. higher than in the park, that the maximum extremes average 0.9° lower and the minimum extremes 6.4° higher and that the mean daily range was 7.3° smaller in the forest.   He also showed that the average wind movement in the forest was only 51 per cent as great as in the park.   Geddes (6) states that where vegetation is rich, the temperature variation during a 24-hour interval is much smaller than in a dry, barren region.   In the latter, all the energy goes to warm the barren surface of the earth, and, as the air is very dry, there is nothing to prevent radiation at night.   In the former, much of the energy is absorbed by the vegetation, and the moisture in the atmosphere arising from the vegetation prevents radiation at night.

Coit and Hodgson (2), in their investigations on the abnormal shedding of young fruits of the Washington Navel oranges, studied the climatic environment in several groves in California, for the purpose of obtaining an integration of all the climatic factors in their effect upon the plant.   They selected the Livingston white cylindrical porous cup atmometer.   They found at their desert station, which was located on the open, bare desert about one-half mile

to windward of the edge of the orchard and many miles to leeward of any irrigated land, that the atmometer lost an average of 94.0 cc. of water daily whereas at their alfalfa station the atmometer lost only 18.5 cc., or only 20 per cent as much.   Thus they show within a half mile in the San Joaquin desert a climatic change of the same magnitude as that between Miami, Florida and Tucson, Arizona.   The same authors state that it is possible to modify climatic conditions in an orchard so as to set crops that are in every way comparable with those produced in much more climatically favored citrus districts.   The means suggested are by heavier and more frequent irrigation, the planting of intercrops, mulching with straw and other materials, protection by means of windbreaks, and a reduction of leaf areas by moderate winter pruning.

PLAN OF INVESTIGATION

The purpose of this investigation was to determine more clearly some of the temperature interrelations between the soil and the atmosphere.   For this purpose temperature readings were taken during the day as well as during the night, especially when it was calm.

The instruments used were standardized chemical thermometers certified by the United States Bureau of Standards and checked by the author, a copper bulb thermometer devised by the author, and electrical resistance thermometers.

Certain stations were selected on the University of Wisconsin farm, located in Madison, Wisconsin and at the Branch of the College of Agriculture, University of California, Davis, California.   The stations were so located that temperature readings could be taken in an uncropped area adjoining a cropped area as well as in the latter.   The cropped areas selected were both on mineral soils and on organic soils at Madison and only on the former at Davis.   The crops were sugar beets, hemp, corn, nursery, oak forests, cabbages, vineyard and orchard.

Air temperatures taken at various heights above the soil surface in these cropped areas when compared with those at the same heights in adjoining uncropped areas where the land was bare, showed a great difference.

The day temperatures were higher (over 6°F. at times) in areas planted to such crops as sugar beets and corn, because these crops permitted the sun's rays free access and because they reduced considerably the velocity of the wind as compared to the uncropped areas.   In the case of a crop such as hemp where the stand was very thick, lower temperatures were obtained during the day at the 6- and 12-inch heights and higher temperatures at the 36- and 60-inch heights as compared to the adjoining uncropped areas because the sun's rays were able to penetrate only a short distance through the hemp whereas the height of the hemp, which was 8 feet, reduced the wind velocity so that the air temperatures in the upper portion of the hemp were higher.

Air temperatures taken before sunrise when there was a slight wind or when

it was calm were higher in the cropped areas than in the uncropped areas. In the case of hemp the air temperatures at heights of 6, 12, 36, and 60-inches were generally from 0.5 to 6.8°F. higher than in the uncropped plots.

Inversions of temperature were found to be the greatest over uncropped plots, whereas the temperatures in the cropped plots were more nearly uniform at the 6-, 12-, 36-, 60-inch heights. On the University Marsh at Madison, Wisconsin (4), an inversion of 4.8°F. was obtained between the 6- and 60-inch heights whereas at the same elevations in the hemp plot there was an inversion of only 0.5° at the same time.   These inversions were found to extend to a height of 96 inches when the greatest height above the soil at which the air temperatures were taken was 168 inches.

The importance of air drainage is well shown in readings obtained in a small nursery at Madison, Wisconsin where the air at the lower end of the nursery was at all heights (6 to 60 inches) from 0.4 to 1.4° lower in temperature than at the upper end which was only three feet higher in elevation.   A similar effect was found in a vineyard on the University Farm at Davis, California where there was a difference of from 1.4 to 2.7°F. between the upper station which was six feet higher in elevation than the lower station.

TEMPERATURE OF SURFACE SOIL

The next phase of this problem investigated was the relation between the temperature of the immediate surface of the soil and the temperature of the air in contact with it.   Various references in literature note that after sunset the surface of the soil becomes cooler than the air in contact with it.   One of these articles (16) makes the following comment:

> After the sun goes down the ground cools rapidly through radiation, and its temperature soon falls below that of the layer of air in contact with it.   As soon as this occurs the surface air begins to lose heat to the ground by conduction.   The air near the ground now becomes cooler than the air above and its density becomes increasingly greater.

Franklin (5) has shown that the temperature of the surface soil depends on the relative humidity, the dryness of the surface layers, and the temperature of the underground layers.   He noted that where the soil had been kept covered during the day, as for instance by a canvas shelter, except in times of cold, rain, sleet, or strong winds and closed at night, the temperature under the shelter was from 2.5 to 7°F. higher than in the open soil. The same author reported results (5) which show that if the surface soil does not freeze, the air minimum over open soil follows the surface soil minimum very closely.

Frost occurrence is more likely on organic than on mineral soils because of the difference between their effects on air temperatures.   This is due to the slower rate of heat conductivity in the case of the organic soils (1), the temperatures of which at the lower depths may be higher than those of the mineral soils.   The mineral soils are good conductors of heat and thus allow the heat

which has been accumulated at the various depths, during the day, to travel to the surface at a greater speed.   Patten (10) has shown that the greatest factor to be considered in heat conductivity is the moisture of the soil.

Bouyoucos and McCool (1) found the surface temperature of the muck soil to be 28°F. and that of the air one inch above the soil surface to be 30.5°F. They obtained this air temperature by having the thermometer in a box similar to the United States Weather Bureau shelter but with the bottom removed. The surface of the mineral soils they reported, however, as being 2.5°F. warmer than the air one inch above.

The problem of obtaining the temperature of the immediate surface of the soil was felt by the author to be of prime importance.   The methods used in the past have been either to lay the thermometer so that the bulb was on the top of the surface of the soil or to cover the bulb with a thin layer of soil.

A large number of readings by both methods were obtained by the author on mineral as well as on organic soils and in only one case was the temperature of the surface soil colder before sunrise than the temperature of the air in contact with it.   This result was obtained on the peat soil, when the surface soil was moist, the air calm, no fog, stars visible, and with frost on the ground. These readings were obtained with mercury thermometers with the bulbs lying on the surface of the peat and $\frac{1}{2}$ inch above the surface.   The air temperature was 26.4°F. whereas the surface temperature was 25.7°F.

It was thought necessary to use some means other than the ordinary type of thermometer to obtain the actual temperature of the immediate surface of the soil because this temperature cannot be obtained by covering the bulb of the thermometer with a thin layer of soil nor, because of the varying thickness of the bulbs, and their length, and of the fact that the contact is just a line contact, can it be obtained by placing the thermometer on the soil surface. Because it was felt to be necessary to obtain temperatures during the same night at various stations rather distant from each other, a portable outfit was necessary.   For this reason mainly it was decided to enlarge the bulb of an ordinary thermometer.

Pieces of well seasoned basswood (fig. 1) measuring 3 by 3 by $\frac{3}{4}$ inches were used.   A groove of sufficient length and depth to accommodate the bulb of a mercury thermometer was cut in each of these blocks.   Dental cement was then placed in this groove, the bulb placed in the cement so that it was completely covered and then a piece of thin copper plate $\frac{1}{40}$ of an inch in thickness was soldered to the bulb so that it was flush with the outside face of the block. By placing this copper plate on the surface of the soil it was felt an accurate record of the temperature could be obtained.   Dental cement was used for making the contact between the copper plate and ordinary mercury thermometer because it is a good conductor and does not contract on hardening. These copper bulb thermometers were standardized against each other and against standard mercury thermometers by the use of cold storage rooms which were maintained at various temperatures, by placing on frozen soil

and covering the bulbs with several layers of burlap, and by placing on hot bottles and covering with burlap so that the graduated stems were exposed for reading.

The copper bulb thermometers as well as the mercury thermometers checked each other closely. The greatest difference at any temperature was 0.6°F. Corrections were made in all readings for these differences so that the temperatures reported are thought by the writer to be comparable with each other.
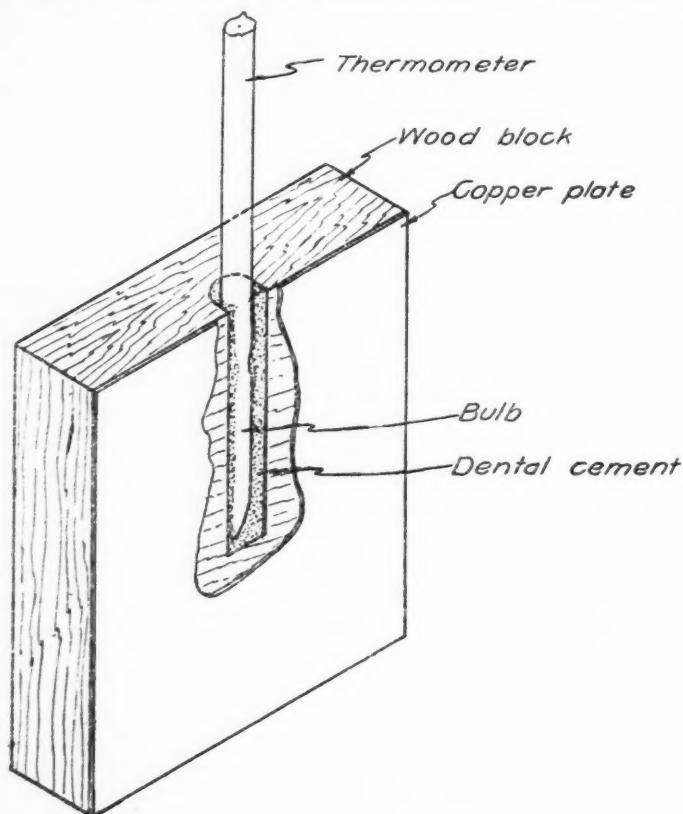
FIG. 1. COPPER BULB THERMOMETER

An attempt was made to use these copper bulb thermometers during sunlight or daylight hours but the radiation from the sky on a cloudy day was found to affect this type of thermometer so that it registered several degrees higher than the mercury thermometer. This affect of sky radiation is no doubt due to the large surface (3 by 3 inches) of the copper bulbs as com-

pared to the small area of the bulb in the mercury thermometers. Eliminating sky radiation by placing the thermometers in a tightly covered box with only the stems extending out resulted in the same temperature being obtained by the use of either type of thermometer.

The copper bulb thermometers could be used, however, during the night and were of greatest value during the hours preceding sunrise. The temperature of the surface soil was found to be lower than that of the layer of air in contact with it (one-half inch above the soil surface) just before sunrise on calm nights. During the fall of 1923 the surface of the peat soil on the marsh was seldom more than 1.5° cooler than the air in contact with it; whereas with mineral soils lying at higher elevations, a difference as high as 4.55°F.
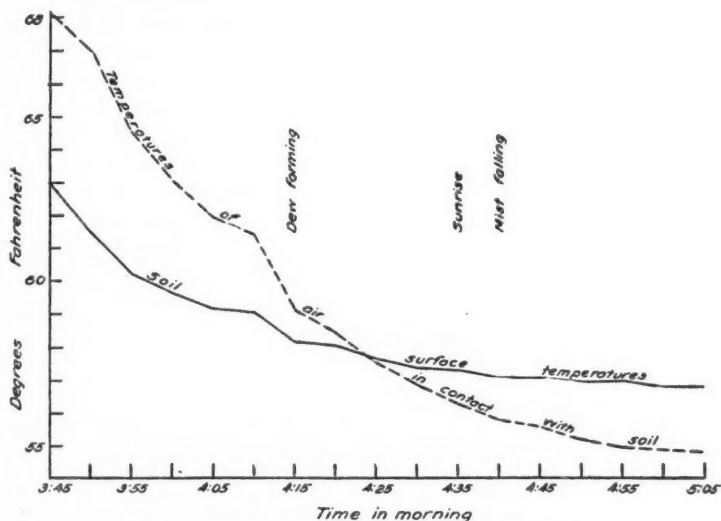
FIG. 2. TEMPERATURES AT STATION 1, JUNE 11, 1924

was obtained, especially when the temperatures were as high as 40 and up to 68°F. When the temperature of the air was close to freezing no great difference in temperature between the soil surface and the air in contact with it was noted.

In the spring of 1924, on calm nights the difference in temperature between the surface of the mineral soils and the air in contact with it was greater than was the case on marsh soils, especially when the humidity of the atmosphere was high. The greatest difference found in the spring of 1924 was at station 1 on a silt loam soil where the surface temperature was 5.1° lower than the air in contact with it. (Fig. 2.)

TEMPERATURE CHANGES BELOW SOIL SURFACE

Soil temperatures taken at depths of 1, 3, and 6 inches by use of mercury thermometers, in general showed no great change during the period of observations before sunrise, which is some cases was two and one-half hours in length. The temperature of the surface soil, however, did change markedly during this period as did the air temperatures taken at various heights.

USE OF ELECTRICAL RESISTANCE THERMOMETERS

A series of sixteen electrical resistance thermometers for obtaining soil temperatures was installed at Davis, California. The soil area selected was alluvial in origin with a loam texture 3 feet deep. The topography was nearly flat. Sixteen plots, 5 meters square were fenced in and a path 2 meters wide was left on the four sides of each plot. The electrical resistance thermometers used were of the bulb type and a sixteen-point automatic recorder was installed in a small one-story frame building with a shingled roof which was 100 feet north of the plots. The thermometers and recorder were supplied by the Leeds Northrup Company. The same amount and quality of wire was used to each thermometer from the recorder and the installation was made overhead so as not to disturb the soil. Cable and conduit were used from the recorder house to the center of five plots. The thermometers were standardized after all connections were made but before they were buried in the center of the plots and were found to be on the average within $\frac{1}{2}°$F. (Plate 1.)

In burying the thermometers a small hole was dug in the center of each plot and the soil from each horizon was separately laid aside. The thermometers were pressed into the north wall of this hold so that their bulbs were in undisturbed soil. The soil was placed back in its original position and slightly tamped in order that each horizon might be put back in as near its original compactness as possible.

Sixteen resistance thermometers were placed at depths varying from 3 inches to 36 inches, one was on the soil surface and one during part of the spring of 1925 was $\frac{1}{2}$ inch above the surface of the soil. From two to six thermometers were placed in each of the five plots.

The recorder was so adjusted that a temperature record from each thermometer was obtained every 15 minutes. The recorder was run continuously day and night except during short intervals when adjustments and repairs had to be made.

A comparison of the electrical resistance thermometers with the copper bulb and mercury thermometers on calm nights showed a difference of 0.5°F.

The temperature of the air $\frac{1}{2}$ inch above the soil surface was never found to be more than 1° higher than that of the surface soil when the electrical resistance thermometers were used. This difference is not so great as was found at Madison by the use of the copper bulb thermometers, but because there were so few calm nights at Davis during the spring of 1925 it cannot be said

at present that the same difference will not be found at the latter place when this work is continued along related lines.

The same effect of sky radiation was found when the electrical resistance thermometers were exposed to the direct sunlight. The author has under consideration a method for the elimination of this factor.

As the thermometers were installed on February 19, 1925 no seasonal changes of temperature can be reported at this time (May 1, 1925). Changes in temperature during a 24-hour interval have in general been found up to the present to extend to a depth of 12 inches in the soil. When the maximum temperature of the surface soil and of the air in contact with it occurred at 2:10 p.m., the maximum temperature at the 12-inch depth did not occur until three hours later. When the minimum temperature of the surface soil and of the air in contact with it occurred at 4:42 and 5:50 a.m. respectively, the minimum temperature at a depth of 12 inches did not occur until five hours later.

A further study which will determine the rate of temperature changes in soils under different conditions is in progress.

SUMMARY

Air temperatures taken at heights varying from 6 to 60 inches above the soil surface were higher during the day (over 6° at times) in cropped areas, such as sugar beets and corn, than in uncropped areas. With a crop such as hemp, where there was a heavy stand, lower temperatures were obtained during the day at the 6- and 12-inch heights and higher temperatures at the 36- and 60-inch heights, as compared to adjoining uncropped areas.

The night air temperatures taken immediately preceding sunrise, when the atmosphere was calm, were higher in the cropped than in the uncropped areas. The greatest differences were found in the hemp, where the air temperatures were at times 6.8° higher.

Inversions of temperature were greatest over the uncropped plots. The highest (4.8°) was over marsh soil.

By the use of an enlarged bulb thermometer, the temperature of the surface soil was found to be lower than that of the air in contact with it just before sunrise on calm nights. Mineral soils, where the drainage conditions were good, showed differences as high as 4.55°; whereas on poorly drained areas, such as peat soils, the temperature of surface was seldom more than 1.5° cooler than the air in contact with it.

During calm weather the temperature of the air in contact with the soil was found to be higher by the use of the copper bulb as well as by the electric resistance thermometers.

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PLATE 1

SOIL TEMPERATURE PLOTS AT DAVIS, CALIFORNIA
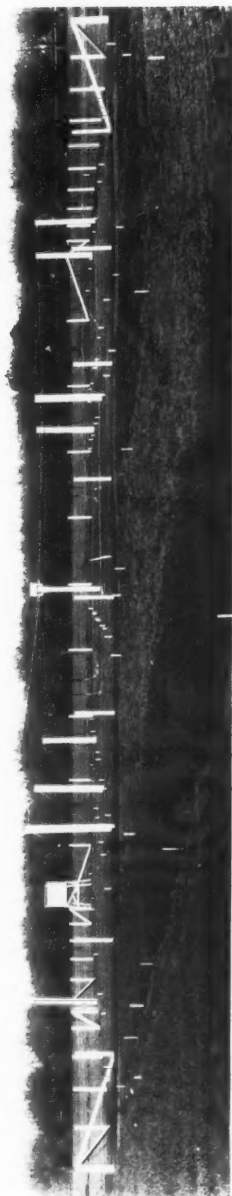
SYNTHETIC CALCIUM SILICATES AS A SOURCE OF AGRICULTURAL LIME: III. A COMPARISON OF THE INFLUENCE OF SYNTHETIC CALCIUM SILICATES WITH OTHER FORMS OF LIME ON THE SOIL REACTION[1]

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INTRODUCTION

As has been pointed out by Page (9) the researches of Bradfield (5) serve as a compliment to the theories of Hissink (7) in regard to the nature of soil acidity and its relationship to the colloidal portion of the soil. The correlation of the degree of saturation of the soil colloids, both clay and humus, and the hydrogen-ion concentration of the soil suspensions has, therefore, meant a distinct advancement in the study of soil acidity. Wiegner (13) has shown that we may consider hydrogen as the general ion adsorptively held on clay particles. From the studies of Ramann (11) on finely divided quartz and on permutites, and from studies by Bradfield (4) on clay suspensions, we may assume that the addition of bases to the soil as an agricultural practice is primarily a neutralization process with some exchange action.

As a natural outgrowth of the studies quoted above, a correlation between the pH of the soil suspensions and so-called "lime requirements" of the soil may be expected within only a given type of soil and even then imperfectly because of the influence of humus. Thus the classification of soils according to the size of particles and the humus content assumes an added significance in the recommendations of the soil scientist to farmers. Again, the measurement of the hydrogen-ion concentration of the soil has only qualitative significance unless one assembles many other data relative to the soil, i.e. content of clay, humus, exchangeable bases, $CaCO_3$. Although methods based on the physico-chemical properties of the soil are as yet far from perfection, the service rendered by Hissink, Gedroiz and others shows the importance of such studies and points to the ultimate significance which they will no doubt assume in the study of the soil.

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Barnette (1, 2) among other investigators, has shown that the ultimate effects on plant growth and on bacterial processes of different liming materials are essentially the same when the materials are added on a chemically equivalent basis. The present study was made during the process of the experiments referred to above and shows the effect of several liming materials, especially synthetic calcium silicates, on the hydrogen-ion concentrations of soil suspensions.

EXPERIMENTAL RESULTS

The method of Gillespie (6) and the apparatus of van Alstine (12) were used in determining the pH of the soil suspensions. The suspensions, made up of 1 part of air-dried soil to 2.5 parts of water, were shaken thoroughly and allowed to stand over night. The hydrogen-ion concentration was determined in the clear or slightly turbid liquid drawn from the top of the settling suspensions. Although it is appreciated that this method is open to objections,

TABLE 1

*Average pH values for the suspensions of the variously treated soils**

TREATMENT	SOIL FROM PLOT N	SOIL FROM PLOT 11A
No lime	5.74	5.59
Ground limestone	6.85	6.55
Di-calcium silicate	7.02	6.61
Hydrated lime	6.88	6.39

* Liming materials on basis of 2000 pounds CaO per acre. All values are the average of seven samples.

it is felt that in the loam soils the buffer action is probably sufficient to prevent any great change in the suspensions through standing over night.

Soils from plots N and 11 A of the New Jersey plots were used in the initial experiments.[3] They were treated with ground limestone, di-calcium silicate, and hydrated lime on an equivalent lime basis of 2000 pounds of CaO per acre and maintained at a constant moisture content, uncropped. Representative samples were taken at intervals. The experiment was carried out in pots under conditions which precluded leaching.

Table 1 gives the average pH values for the suspensions of the variously treated soils. Figure 1 gives the average changes in pH values of suspensions of the variously treated soils as compared with the original soils. The values for the soils receiving the different materials on an equivalent lime basis were near enough to come well within the limits of error of this type of experimentation. The time intervals were 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, and 13 weeks after the date of application.

From these results it may be seen that the different liming materials affected

[3] These soils are described in a previous publication (1).

the hydrogen-ion concentration of the soil suspensions in much the same manner. The hydrogen-ion concentrations of the soil suspensions showed a progressive increase with time (fig. 1), the changes were similar for all the different materials, and the pH values were practically the same for the various time intervals and for the three materials. The curves in figure 1 are thus typical of the changes produced by the prolonged contact of the soil with a liming material. The development of carbonates, nitrates, and sulfates, together with the fixing processes, is responsible for these changes. These
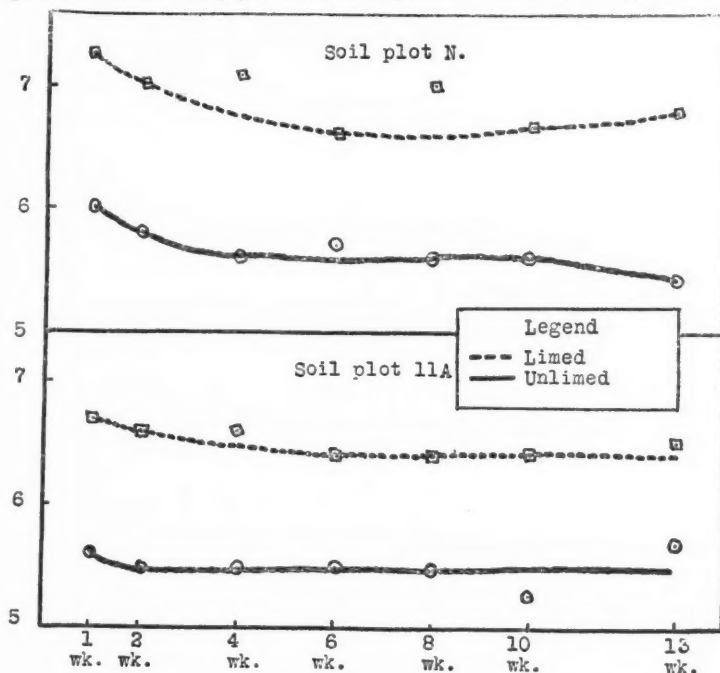
FIG. 1. CHANGE IN HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS OF LIMED AND UNLIMED SOILS WITH TIME

results are in accord with the findings of Hoagland and Christie (8). More recently Bobko and Druschinin (3) have shown that the buffer system in extracts of humus-free limed soils (soils containing $CaCO_3$) is essentially the system: free CO_2-HCO_3; whereas in those of unlimed soils, "podsoils," humus-rich soils, and "torfs" the system is controlled by an acid having a larger dissociation constant than carbonic acid.

Soils from plot 11 A, from an acid Sassafras loam, and from a well limed Penn loam were used in obtaining the results given in table 2. The same

methods were employed as above and the soils were treated similarly save that they were cropped. From this table and from figure 2, the influence of increasing amounts of lime as "limosil" on the hydrogen-ion concentration of soil suspensions may be seen. The well-limed Penn loam soil showed little change of hydrogen-ion concentration with the addition of increasing amounts of "limosil." The buffer action in this soil was exceptionally pronounced. When it is recalled that "limosil" is a mixture (calcium silicate, for the greater part, and lime), it may well be imagined that the maximum solubility of the compounds at the CO_2 tension present was reached with the smallest application with the resulting more or less constant pH (5). In the acid soils (plot

TABLE 2

The influence of applications of the various liming materials on the hydrogen-ion concentration of three soils under crops

TREATMENT	EQUIVALENT APPLICATION OF CaO PER ACRE	SOIL FROM PLOT 11A					SASSAFRAS LOAM					PENN LOAM				
		After 1 month	2 months	8 months	11 months	Average	After 1 month	2 months	8 months	11 months	Average	After 1 month	2 months	8 months	11 months	Average
	pounds	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
No lime......		4.85	4.9	4.8	4.05	4.65	5.2	5.95	5.45	4.45	5.26	7.05	6.95	6.6	6.4	6.75
Limosil.....	500	6.10	5.3	5.0	5.3	5.42	6.7	6.6	5.9	5.5	6.17	7.1	7.1	6.4	6.7	6.83
	1,000	6.8	5.9	5.5	5.3	5.75	6.8	6.2	5.7	5.8	6.12	7.3	6.9	6.7	6.8	6.93
	2,000	6.5	6.2	5.8	5.85	6.09	6.9	6.5	5.95	6.05	6.35	7.25	7.4	7.4	6.9	7.24
	4,000	6.9	6.7	6.3	6.4	6.57	7.2	6.7	6.2	6.5	6.65	7.3	7.3	7.5	7.2	7.32
Ground limestone.......	2,000	6.3	6.6	5.9	5.9	6.17	7.2	6.5	6.0	6.1	6.45	7.1	7.3	7.0	6.7	7.03
Hydrated lime........	2,000	6.5	6.1	5.5	5.9	6.00	6.8	6.2	6.0	5.9	6.23	7.3	7.3	7.4	6.8	7.20
Di-calcium silicate.....	2,000	6.4	6.6	6.1	5.9	6.25	6.8	6.5	6.3	5.9	6.38	7.1	7.1	7.4	6.9	7.13

11 A and Sassafras loam) an entirely different condition prevails; here the soils exhibit the typical buffer curves of acid loam soils.

The four liming materials, "limosil," ground limestone, hydrated lime, and di-calcium silicate, applied on a chemically equivalent lime basis (2000 pounds CaO per acre) produced substantially equal changes in the hydrogen-ion concentration of the soil suspensions. The pH values for the different soils naturally varied.

The change of the hydrogen-ion concentration with time of contact of the materials is similar to that observed in the first series; the limed Penn loam showed the least increase. Figure 3 gives the average results for the three soils treated with the four different materials on an equivalent lime basis. The gradual decrease with elapse of time is noted.
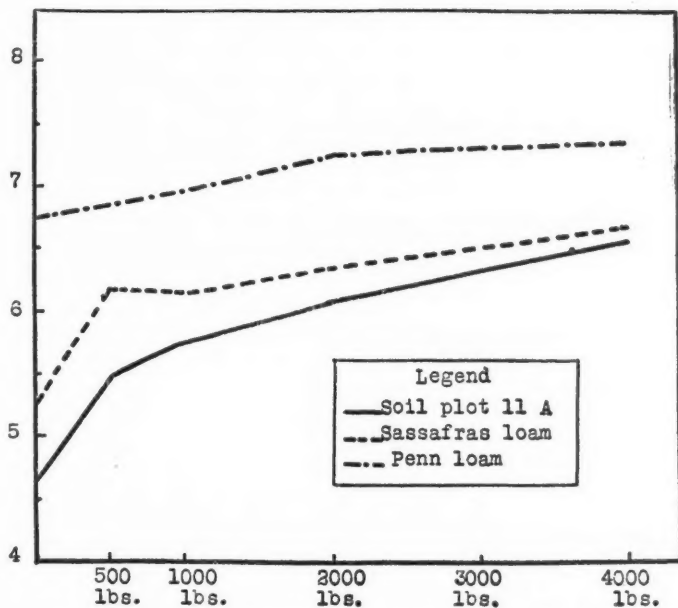
FIG. 2. RELATIONSHIP BETWEEN INCREASING APPLICATION OF LIME AS "LIMOSIL" AND HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS
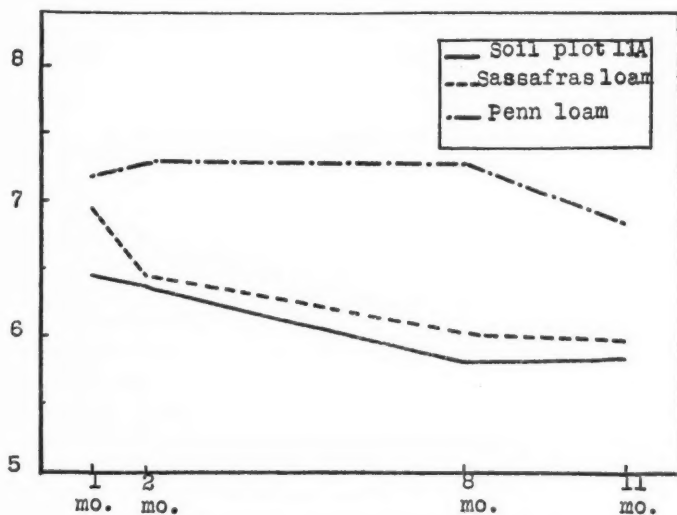
FIG. 3. CHANGE OF HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS TREATED WITH VARIOUS LIMING MATERIALS ON A SIMILAR CaO BASIS

In order to determine the influence of cropping on the hydrogen-ion concentration of soil suspensions a series of pots was started in which one set was treated with five different liming materials on an equivalent lime basis and cropped, and in which the other set was left uncropped. Samples of the soil were taken at intervals and the pH values determined by the colormetric method. Samples were taken at 4-week intervals after the second week, ending with the twenty-fifth week. The Sassafras loam used in the experiments above, and an acid Elkton silt loam were used. These soils were cropped first to buckwheat and then to soybeans. The averaged pH values for the seven samples of the cropped and of the uncropped series taken at various intervals are given in table 3.

From this series it may be assumed that there is no definite influence on the hydrogen-ion concentration of soil suspensions due to cropping. This is in accordance with the findings of Pierre (10).

TABLE 3

The effect of cropping on the pH values of soil suspensions

TREATMENT	WATER EXTRACT OF SASSAFRAS LOAM*		WATER EXTRACT OF ELKTON SILT LOAM†	
	Cropped	Uncropped	Cropped	Uncropped
	pH	pH	pH	pH
No lime	4.91	4.81	4.79	4.71
Calcium oxide	6.17	5.94	5.76	5.99
Hydrated lime	6.11	6.04	5.83	5.89
Ground limestone	6.07	5.85	5.70	5.89
Di-calcium silicate	6.17	5.97	5.81	6.41
"Limosil"	6.20	6.18	5.80	6.11
CaO ≏ free CaO in limosil	5.10	4.99	4.95	5.11

* Liming materials on the basis of 2000 pounds CaO per acre.

† Liming materials on the basis of 3200 pounds CaO per acre.

GENERAL DISCUSSION

The changes in the soil system brought about by the addition of a liming material are so complex that an extensive comparison of the different materials is seldom made. An investigator must limit his work to one material in order to gather enough data conclusively to defend his findings. Thus on the system: acid soil + ground limestone, we find many studies with regard to the influence of the properties of the limestone and of its fineness of divisions, which together with the action in various types of soil assist in clarifying the general problem. One may conclude from numerous similar studies that although the initial effects of the different liming materials, when applied on a practical basis to soils deficient in lime, may be different, the final effects are the same whether the lime is applied as carbonate, hydrate, or silicate so long as the application is practical. Naturally, such a statement applies only

to materials which are finely enough divided to give a comparable surface action.

Thus, studies with plants, with microbiological processes, and with the reaction of acid soil indicate that when the different liming materials are applied on a chemically equivalent and a practical basis, final reaction with the soil is the same. The researches of MacIntire and his associates, together with numerous other workers show that a formation of silicates and a combination with humus of basic materials take place. It must be recalled also that the formation of carbonates, of sulfates, and of nitrates in the soil is apparently dependent upon the available base (Ca, Mg, etc.) in a soil. From this viewpoint, the synthetic calcium silicates studied are not different in their action from other common liming materials.

SUMMARY

From studies of the hydrogen-ion concentration of soils treated with different liming materials, the following observations were made:

1. Chemically equivalent and practical applications of calcium carbonate, hydrated lime, dicalcium silicate, and "limosil" (mixture of mono-calcium silicate and CaO) produced, within the limits of experimental error, equal changes in the hydrogen-ion concentrations of soils to which they were added.

2. There is a progressive increase in the hydrogen-ion concentration of soils treated with liming materials following the date of application.

3. The measurement of the pH values for two acid loam soils treated with increasing applications of "limosil" showed typical buffer curves for loam soils; whereas the pH values for a well limed Penn loam (free from humus) showed little increase with increased applications of "limosil."

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A STUDY OF THE ROOT-NODULE BACTERIA OF WOOD'S CLOVER (DALEA ALOPECUROIDES)[1]

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Wood's clover is becoming recognized as a valuable annual legume for use as a green manure. Since it is a newly cultivated legume, the question of the relation of its root-nodule bacteria to the already recognized groups of nodule bacteria is of decided importance. Schneider (2) reported in 1892 that the bacteria from nodules of Dalea were different from the others, which included the bacteria of many common legumes, then under study.

CROSS-INOCULATION STUDIES

In order to place in the proper group the bacteria of a legume not previously studied, it is necessary to make cross-inoculation tests with typical members of the established groups. No other method of placing an unknown organism in a group has yet been devised. It is evident that the cultures used for such experiments must be free from all root-nodule bacteria except those common to the legume or group to be studied. The presence of only a few organisms of any other legume in the culture applied would give erroneous results in such crosses.

The cross-inoculation results reported in table 1 were obtained from seven experiments in which three to five jars, containing from 100 to 200 plants per jar, were used for each strain of the organism tested.

Wood's clover nodule bacteria have not been found to cross inoculate with the eleven groups represented in these experiments. From these results there has been established a new group for this legume. It is necessary to use the bacteria from Wood's clover nodules for nodule production on this legume. It is quite probable that some other nodule bacteria belong to this group, but as yet they have not been found. A very large number of cross-inoculation groups undoubtedly exist and future study will add to those recognized at present.

FIELD OBSERVATIONS

Cross-inoculation studies under controlled conditions are the only reliable procedure for determining group relationships. Under field conditions one

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may sometimes test out the controlled experiments. Bacteria-free Wood's clover seed grown in field plots where a number of common legumes had previously grown showed no inoculation. Further field seedings where vetch, lupine, alfalfa, red clover, and sanfoin soil had been applied showed no inoculation on the Wood's clover.

A field seeding will occasionally show some inoculation even with a new legume. Such observations, unless thoroughly investigated, are very likely to lead to wrong conclusions. This chance inoculation has been traced to nodule bacteria carried on the seed. Much variation has been found in different lots of seed, some lots carrying too few bacteria to be valuable for inoculation but too many for cross-inoculation studies without complete sterilization of the seed. Some yet undiscovered wild legume may be inoculating Wood's clover in occasional cases.

TABLE 1

Cross-inoculation experiments with Wood's clover (Dalea alopecuroides)

KIND OF PLANT	KIND OF BACTERIA	NODULE FORMATION
Dalea	Alfalfa, strains 31, 32, 33	No nodules
Dalea	Clover	No nodules
Dalea	Pea, strains 3, 4, 10, 10A, 15, 20	No nodules
Dalea	Bean	No nodules
Dalea	Wisteria	No nodules
Dalea	Lupine	No nodules
Dalea	Locust	No nodules
Dalea	Lead Plant	No nodules
Dalea	Hog peanut	No nodules
Dalea	Cowpea	No nodules
Dalea	Soybean	No nodules
Alfalfa	Dalea	No nodules
Sweet clover	Dalea	No nodules
Dalea	Not inoculated	No nodules
Dalea	Dalea	Large abundant nodules on all plants

An example of the benefit to be derived from reinforcing any natural inoculation carried on the seed by applying inoculation appears in plate 1. Plate 2 shows the roots and nodules of Wood's clover inoculated with the culture under study.

NITROGEN AND PROTEIN CONTENT OF WOOD'S CLOVER

Four samples of Wood's clover of spring seeding, taken July 30 and September 4, showed an average nitrogen content of 2.66 per cent, which would equal 16.62 of protein on a water-free basis provided all the nitrogen was present in the form of protein. Six samples of plants 12 inches tall from a summer seeding averaged 4.08 per cent nitrogen or 25.80 per cent protein. Al-

though a high nitrogen content is not uncommon with young plants, this is very high for plants 54 days old. Hughes (1) has reported a protein content at the time of plowing under of 15 per cent, and, in younger plants, of 20 per cent.

SOME CHARACTERISTICS OF WOOD'S CLOVER NODULE BACTERIA

The root-nodule bacteria of Wood's clover were isolated from nodules taken from plants grown in the field. Purity was determined by the accepted methods, which included growth in litmus milk and inoculation on potato slopes. These organisms grew rapidly on mannitol agar. Abundant slime of a non-sticky nature was produced. Growth was opaque and resembled that of the nodule bacteria of clover more than that of alfalfa. The shape of the organisms varied with different sugars: with some they were short rods; with others large oval cells. Mannitol gave organisms 3.3μ by 1μ; glucose, 1.7μ by 0.83μ; sucrose, lactose, and xylose, 1μ by 0.8μ; plain and mannitol milk, 3–4μ by 1μ. Motility was observed in mannitol and glucose solutions. The largest number of motile organisms was found with the mannitol solution. On plain agar the organisms were very motile at 24 hours. The usual irregular bacteriod shapes were seen. When stained by the Plimmer-Paine method peritrichic flagella were demonstrated.

GROWTH ON POTATO SLOPES

This organism shows more ability to grow on potato than most of the other known root-nodule bacteria. Cultures replated and many times transferred still show a slight watery growth when placed on potato slopes. It is not dissimilar in appearance to a heavy inoculation when the latter is observed soon after being placed on the potato. The nature of the potato slopes appears to influence the amount of growth that results. On some lots very little growth of root-nodule bacteria occurs, whereas on others much growth appears.

SERUM ZONE FORMATION, REACTION, AND REDUCTION IN MILKS

Growth in litmus milk gives a very deep serum zone after several weeks. Zone formation starts very rapidly as compared with other root-nodule bacteria, often showing in 2 days, and progressing to a point where it equals one-half or more of the original volume of the milk. The reaction in the serum zone is at first alkaline, then becomes neutral and later acid. The litmus becomes entirely reduced in the solid portion of the milk. Growth appears to take place at the surface and throughout the entire depth of the milk. In brom-thymol-blue milk, after several weeks a condition similar to that found in the litmus milk occurred, with the reaction in the serum zone slightly acid. In plain milk a very deep serum zone is also evident. A typical serum zone was produced in litmus milk containing 5 per cent mannitol and in one with 5 per cent sucrose, and considerably later in that to which the 5 per cent dex-

trose was added. This serum-zone formation in milk is typical of the bacillus type of root-nodule bacteria.

GROWTH WITH DIFFERENT SUGARS

The bacteria from this legume grew very rapidly with all the sugars except lactose. The increase in growth with that sugar was quite remarkable after a slow start—at 12 days it was equal to both sucrose and xylose. The glucose media contained peptone and beef extract, which made it not strictly comparable with the other sugars. The exceptionally rapid growth of some strains of nodule bacteria has been attributed to their being recently isolated from the plant or in recent contact with the soil. Although such an effect is often noted with some strains, with others a very great stimulation in growth results from repeated culturing, as judged on one kind of media. The Dalea

TABLE 2

Relative growths with different sugars

SUGAR		GROWTH AFTER			
		2 days	12 days	22 days	42 days
Mannitol		++++	++++	++++	++++
Mannitol	+CaCO₃	+++++	+++++	++++	++++
Sucrose		++++	++++	++++	++++
Sucrose	+CaCO₃	++++	+++	+++	+++
Lactose		+	++++	+++	+++
Lactose	+CaCO₃	++	+++	+++	++++
Xylose		+++	+++	+++	++
Xylose	+CaCO₃	++++	++++	+++	+++
Glucose*		+++++	+++++	++++	++++
Glucose*	+CaCO₃	+++++	++++	++++	++++

* Peptone and beef extract added.

+ indicates scant growth; ++, fair growth; +++, good growth; ++++, excellent growth; +++++, heavy growth.

organism has so far never failed to give a very rapid growth under the conditions of this study. The strains isolated may have been fast growers only and further search may result in the isolation of slow growers.

CONCLUSIONS

1. Wood's clover (*Dalea alopecuroides*) does not possess root-nodule bacteria in common with any other legume group, consequently it is placed in a group by itself for inoculation purposes.

2. The nitrogen content of this annual legume is very high and compares favorably with other legumes.

3. The root-nodule organism of this legume produces in milk a serum-zone

characteristic of the bacilli group.   The organism is motile and its flagella are pertrichous.

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PLATE 1

APPLIED INOCULATION COMPARED WITH NATURAL INOCULATION

Plants on left grown from seed inoculated with pure culture. Plants on right not inoculated; some inoculation was carried on the seed, as rows of sterilized seed showed no nodules.

Complete inoculation

15 percent inoculation

Dalea alopecuroides
(Wood's Clover)
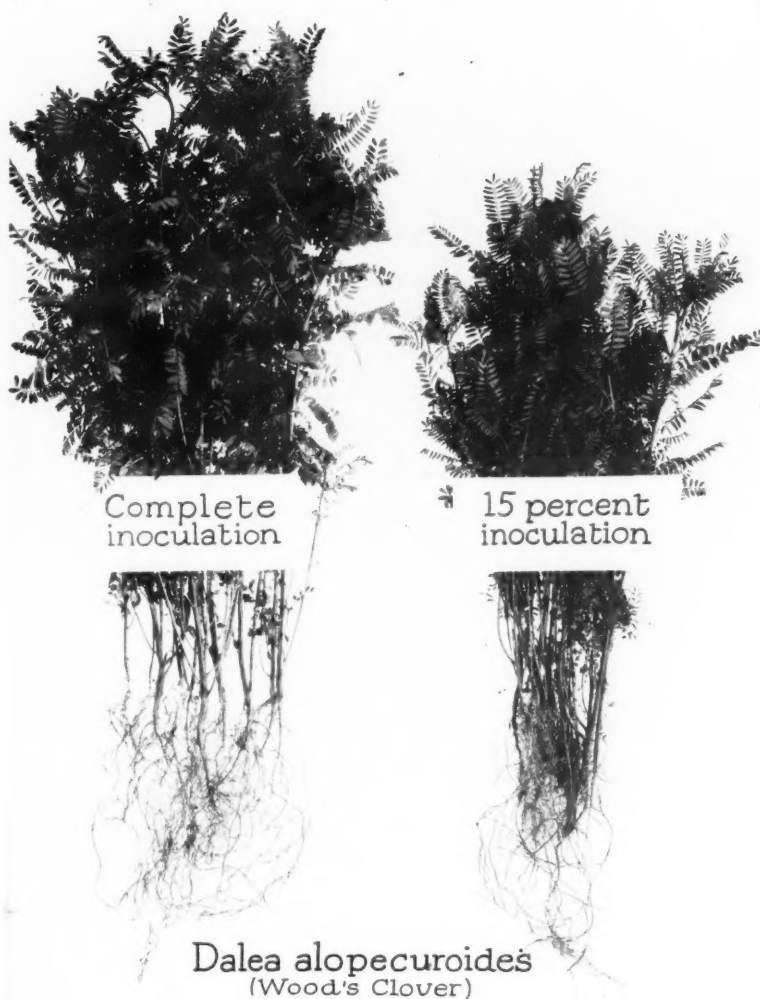
PLATE 2

Fig. 1. Wood's Clover Roots and Nodules

About twice natural size

Fig. 2. Wood's Clover Root-nodule Bacteria Showing Flagella Stained by the Plimmer and Paine Method

Magnification 1425. Made by Colmer
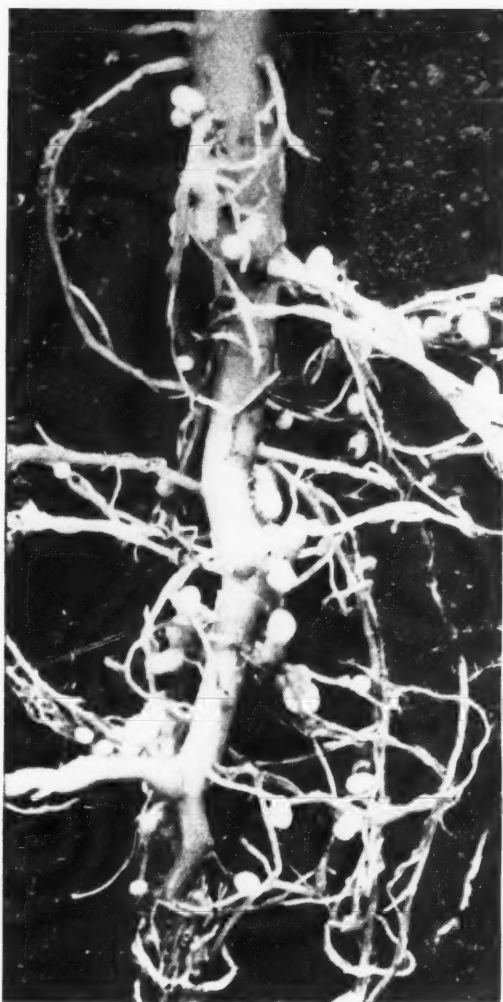
FIG. 1

FIG. 2

THE ASSIMILATION OF PHOSPHORUS FROM PHYTIN BY OATS[1]

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The problem of keeping ample amounts of phosphorus in the form available for growing plants will be solved in a large measure by combining as much as possible of the total soil phosphorus into the active organic matter of the soil. The phosphorus compounds of actively decomposing organic matter are very rapidly liberated in a soluble form by soil bacteria. Organic phosphorus is not subject to conversion into iron and aluminum phosphates so readily as when applied in the inorganic forms.

The following quotation from Dr. C. M. Hutchinson (3) is of interest in this connection:

There has been much conflict of opinion amongst soil bacteriologists as to the solubilizing action of soil bacteria upon insoluble phosphates, but there can be no doubt that under certain conditions such action takes place. Work in this laboratory on this subject during the past 3 years has demonstrated conclusively the possibility of considerable and continuous solubilization of tricalcic phosphate, such as apatite, purely by bacterial action under controlled conditions in the presence of sufficient organic matter.

The rapidly increasing use of green manures offers an opportunity to take advantage of the feeding capacity of these crops by supplying them with inexpensive forms of phosphorus and thereby enabling them to build up their phosphorus content and to enhance their value for the succeeding crops. A new method of mixing inorganic phosphates with fermenting green manures is being studied in some countries. This has proved highly profitable in India. Much of the phosphorus in such a mixture would seem to be in the bacterial cells. If this is true, then another form of organic phosphorus in soil commands study. In the fermenting of high cellulose-containing materials in composts, phosphorus might be used to advantage, being converted into organic compounds by the bacteria and later being available upon their decomposition.

This opportunity to increase the content of organic phosphorus in green manures and to liberate that element from inorganic phosphates where the

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same green manures nitrify after being plowed under, should be taken into consideration in any proper cropping system.

In taking up the study of the assimilation of different forms of organic phosphorus, phytin was first chosen because of its prevalence in crop residues.

LITERATURE CONCERNED WITH PHYTIN

Nagaoka (4) who investigated the relative values of organic phosphates from animal and vegetable origin, found those of animal origin better for plant growth.

Aso and Yoshida (2) made comparative studies of vegetable substances containing phosphorus. Their results are given in table 1. Compared with lecithin and nucleo-protein, phytin was an intermediate source of phosphorus.

Rose (6) grew lupin seedlings in different solutions for 6 to 9 days and measured the root elongation every 3 days. As the addition of phytin did

TABLE 1

Comparative yields of grain with organic and inorganic phosphorus

SUBSTANCE	HEIGHT	DRY MATTER	GRAIN
	cm.	gm.	gm.
Lecithin....................................	20.4	11.7	4.5
Phytin.....................................	11.1	2.5	0.5
Nucleo protein.............................	9.3	2.3	0.0
Tricalcium phosphate.......................	21.0	9.9	3.2
Disodium phosphate........................	15.9	6.0	1.8
Iron phosphate.............................	9.6	3.5	0.8
Aluminum phosphate.......................	10.2	3.0	0.5
Check......................................	7.8	1.4	0.0

not cause any stimulation of growth, it was thought that the acid radical might not be conducive to plant growth. The same author gave the ratio of carbon: phosphorus in phytin as 6:6. He considered phytin as more than a reserve material, and expressed the belief that, in all probability, it entered into the synthesis of phospho-proteins and lipoids.

Thompson (7) isolated phytin from rice bran and unpolished rice, but failed to obtain more than a trace from polished rice.

Patten and Hart (5) found most of the phosphorus of wheat bran to be organic and pointed out that phytin was widely distributed in the vegetable kingdom.

Anderson (1) has made the most important researches on phytin. His work includes studies on the phytin content of oats, of corn, and of wheat bran, and many papers dealing with the composition of the organic phosphorus compounds of these and other substances. It is important to note the occurrence of phytin and related inosite hexaphosphate ($C_6H_{18}O_{24}P_6$) and other similar organic phosphorus compounds in farm crops.

Many data are available bearing upon the high phosphorus requirement of, and its stimulation to, soil bacteria. These references indicate the building of inorganic phosphorus into organic phosphorus by bacteria living in the soil. Such phosphorus is temporarily removed from solution but is easily returned to solution by bacterial decomposition of bacterial bodies. How much competition there may be between bacteria and higher plants for phosphorus in soils is not known. This probably would cease to be important if the nitrogen bacteria were encouraged to predominate in the soil flora.

EXPERIMENTAL

The pot-cultures were conducted in one-gallon jars. Clean white quartz sand that had been thoroughly leached with dilute hydrochloric acid and then washed free from acid with nitrogen-free distilled water was used. In all series except 29–56, additions of pure reprecipitated calcium carbonate and of magnesium sulfate supplied the calcium and the magnesium. Dolomite supplied both these elements for series 29–56. Kainit, free from phosphorus, was added as a potassium carrier for series 29–56, and potassium sulfate was employed for that purpose in the other series. Nitrogen was added as ammonium nitrate. The plant-food applications were made as suggested by Hopkins and Pettit.

PHOSPHORUS ADDITIONS

Phosphorus was supplied in the forms given in the list below.

Material	Phosphorus per cent
Phytin	19.20
Crude phytin	12.96
Alfalfa, third cutting	0.168
Leached alfalfa	0.067
Tennessee rock phosphate	12.120
Tricalcium phosphate	14.600

The phytin is termed pure phytin in this work to distinguish it from the cruder product. It was apparently a mixture of calcium phytate and phytic acids.

OATS SERIES 29–56

The object of this preliminary series 29–56 was to compare the yields of oats grown with organic and inorganic phosphates. Three different forms of phosphorus were used; namely, rock phosphate, pure phytin, and crude phytin. Through an error in the analysis, different amounts of phosphorus in the different forms were added. Although the treatments are not identical, they will give an idea as to the relative availability of the different forms of phosphorus. All pots excepting 29 and 30 received 28 gm. of dolomite, 1.75 gm. of kainite and soluble nitrogen in the form of ammonium nitrate. Seven kilograms

of clean quartz sand was weighed out and the dolomite, kainite, and the phosphorus-bearing substances were added and thoroughly mixed before being placed in the pots. After being wet with distilled water, a sufficient number of oat seeds was planted to insure a stand, and when the seedlings were a few days old they were thinned to ten plants per pot. According to the analysis of the oat grains, not more than 0.9 mgm. of phosphorus was added to each pot in the seed. In table 2, the treatments are expressed in milligrams of

TABLE 2

Treatments and yields of oats, series 29–56

POT NUMBER	PHOSPHORUS TREATMENT PER POT	WEIGHT CROP	WEIGHT GRAIN
	mgm.	*gm.*	*gm.*
Checks			
29–30	None	0.8	. . . . .
31–32	All but P	0.8	. . . . .
Rock phosphate series			
33–34	212	2.7	0.015
35–36	424	3.9	0.133
37–38	848	9.8	0.118
39–40	2545	18.9	0.207
Pure phytin series			
41–42	221	22.9	6.743
43–44	442	18.7	4.377
45–46	883	17.8	2.375
47–48	2650	16.7	1.408
Crude phytin series			
49–50	175	24.2	4.598
51–52	351	25.3	5.116
53–54	702	20.4	3.197
55–56	2106	6.9	0.263

phosphorus per pot and the yields in grams per pot. All yields of the two duplicate pots are averaged in the tables.

It will be observed that the rock phosphate even in large amounts produced scarcely any grain. This may be largely due, however, to the fact that the oats matured in January. With the pure phytin, the minimum treatment produced the maximum yield. With crude phytin, the 351-mgm. treatment produced the maximum yield. The experiment tends to show two points: first, the organic phosphorus as phytin is much more readily assimilated by

plants than the phosphorus from rock phosphate; and second, phytin supplied in large amounts is deleterious as judged by the yields. By considering the amount of phosphorus supplied in the two forms of phytin it will be seen that the yields are very close together and that 220 mgm. of phosphorus per pot as phytin seemed to produce about the maximum yield. Larger amounts than this were deleterious. A toxic effect from the crude phytin was noticeable during growth.

TABLE 3

Percentage and total weight of phosphorus in grain and straw on oats series 29–56

POT NUMBER	PHOSPHORUS TREATMENT PER POT	GRAIN P	STRAW P	GRAIN P	STRAW P	CROP TOTAL P
	mgm.	per cent	per cent	mgm.	mgm.	mgm.
			Checks			
29–30	None	....	0.065	.....	0.53	0.53
31–32	All but P	....	0.060	.....	0.48	0.48
			Rock phosphate series			
33–34	212	0.65	0.063	0.09	1.69	1.78
35–36	424	0.47	0.065	0.62	2.45	3.07
37–38	848	0.63	0 082	0.74	7.94	8.68
39–40	2545	0.60	0.125	1.24	23.36	24.60
			Pure phytin series			
41–42	221	0 62	0.295	41.8	47.6	89.4
43–44	442	0.64	0.815	28.0	116.7	144.7
45–46	883	0.74	1.390	17.6	214.4	232.0
47–48	2650	0.85	1.940	11.9	296.7	308.6
			Crude phytin series			
49–50	175	0.53	0.164	24.4	32.1	56.3
51–52	351	0.59	0.404	30.2	81.5	111.7
53–54	702	0.64	0.764	20.4	131.4	151.8
55–56	2106	0.73	0 992	1.9	65.8	67.7

Table 3 which gives the analytical results of this series, shows that there is an increase in the percentage of phosphorus for increases in phosphorus applications in both grain and straw, and that this holds for both forms of phytin and also for the rock phosphate in the grain. The grain shows only a small amount of tolerance for phosphorus, whereas the straw shows a large amount.

Plate 1 shows the growth of oats on the rock phosphate and on the crude phytin series.

Figure 1 shows graphically the percentages of phosphorus in the grain and straw.

OATS SERIES 101–184

This second oats series 101–184 comprising 84 pots, was much larger than the first. In this series the phosphorus was supplied in five different forms, each being added in four different amounts. In the first five series only one form of phosphorus was added to a series, whereas in the last five series, two kinds—one organic and the other inorganic—were combined in each series. The object of the first five series was mainly to check the combination series. An attempt was made to confine the applications to such amounts that pot limitation would not be a factor, but in some of the higher treatments it
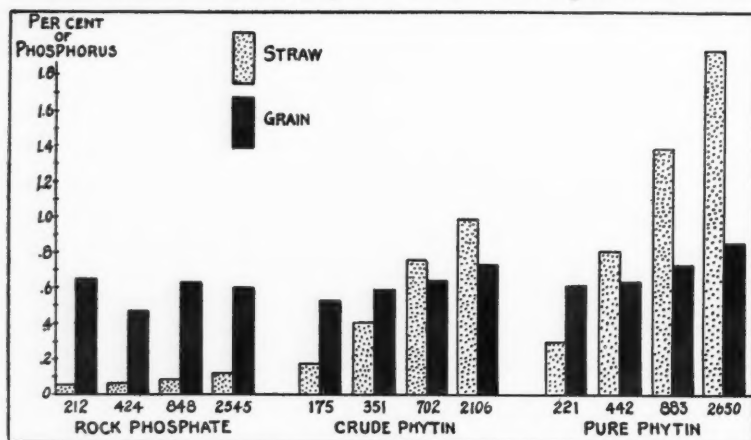
FIG. 1. COMPARATIVE PERCENTAGES OF PHOSPHORUS IN OAT STRAW AND GRAIN

The figures at the base of the double columns are the milligrams of phosphorus applied per jar.

did enter. The applications of phosphorus on the single series were made in the following forms and amounts per pot:

> *Tricalcium phosphate*—28, 55, 110, and 330 mgm.
> *Rock phosphate*—220, 440, 880, and 1320 mgm.
> *Alfalfa*—11.9, 23.8, 35.7, and 47.6 mgm.
> *Leached alfalfa*—4.9, 9.8, 14.7, and 19.6 mgm.
> *Crude phytin*—11.9, 23.8, 35.7, and 47.6 mgm.

The alfalfa and leached alfalfa were applied at the rate of 7, 14, 21, and 28 gm. per pot respectively, thus adding to each pot a comparative amount of organic matter.

The combination series were made up as follows:

Alfalfa plus rock phosphate. To each pot of an alfalfa series was added 880 mgm. phosphorus as rock phosphate.

TABLE 4

Treatment and weights of grain and total crop on oats series 101–144

POT NUMBER	PHOSPHORUS TREATMENT PER POT	WEIGHT CROP	WEIGHT GRAIN
	mgm.	*gm.*	*gm.*
	Checks		
101–2	None	0.9	....
103–4	All but P	1.6	....
	Tricalcium phosphate series		
105–6	28	2.0	0.09
107–8	55	2.0	0.12
109–10	110	3.5	0.69
111–12	330	4.6	1.17
	Rock phosphate series		
113–14	220	7.0	1.08
115–16	440	10.3	2.15
117–18	880	15.5	3.59
119–20	1320	16.2	4.84
	Alfalfa series		
121–22	11.9	6.6	0.96
123–24	23.8	7.0	1.73
125–26	35.7	10.5	2.26
127–28	47.6	8.5	2.48
	Leached alfalfa series		
129–30	4.9	1.4	....
131–32	9.8	1.2	....
133–34	14.7	1.1	....
135–36	19.6	1.1	....
	Crude phytin series		
137–38	11.9	3.7	0.48
139–40	23.8	6.3	1.09
141–42	35.7	9.5	2.04
143–44	47.6	15.7	4.07

Leached alfalfa plus rock phosphate. To each pot of a leached alfalfa series was added 880 mgm. of phosphorus as rock phosphate.

Phytin plus rock phosphate. To each pot of a phytin series was added 880 mgm. of phosphorus as rock phosphate.

Rock phosphate plus alfalfa. To each pot of rock phosphate series was added 21 gm. of alfalfa containing 35.7 mgm. of phosphorus.

Rock phosphate plus phytin. To each pot of a rock phosphate series was added 35.7 mgm. of phosphorus as phytin.

In order to allow ample time for the decomposition of the alfalfa and the establishment of an equilibrium in the sand, the fertilizers were mixed with the sand, potted, and wet down on December 28 with a liberal amount of soil infusion.   On January 20, 16 kernels of oats were planted in each pot and

TABLE 5

Treatment and yields on combination oat series 145–184

POT NUMBER	PHOSPHORUS TREATMENT PER POT	WEIGHT CROP	WEIGHT GRAIN	THEORETICAL YIELD
	mgm.	*gm.*	*gm.*	*gm.*
Alfalfa plus rock phosphate				
145–46	Alfalfa 11.9, R. P. 880	13.6	3.72	4.55
147–48	Alfalfa 23.8, R. P. 880	15.7	4.10	5.32
149–50	Alfalfa 35.7, R. P. 880	12.8	3.56	5.85
151–52	Alfalfa 47.6, R. P. 880	11.0	2.85	6.07
Leached alfalfa plus rock phosphate				
153–54	Leached alfalfa 4.9, R. P. 880	10.6	2.21	3.59
155–56	Leached alfalfa 9.8, R. P. 880	7.0	1.79	3.59
157–58	Leached alfalfa 14.7, R. P. 880	7.6	2.06	3.59
159–60	Leached alfalfa 19.6, R. P. 880	7.0	1.76	3.59
Phytin plus rock phosphate				
161–62	Phytin 11.9, R. P. 880	18.6	4.91	4.07
163–64	Phytin 23.8, R. P. 880	17.0	4.81	4.68
165–66	Phytin 35.7, R. P. 880	21.2	6.07	5.63
167–68	Phytin 47.6, R. P. 880	19.1	5.45	7.66
Rock phosphate plus alfalfa				
169–70	R. P. 220, alfalfa 35.7	12.6	3.74	3.34
171–72	R. P. 440, alfalfa 35.7	15.4	3.48	4.41
173–74	R. P. 880, alfalfa 35.7	12.8	3.56	5.85
175–76	R. P. 1320, alfalfa 35.7	15.5	4.48	7.10
Rock phosphate plus phytin				
177–78	R. P. 220, phytin 35.7	15.7	3.80	3.12
179–80	R. P. 440, phytin 35.7	16.0	3.88	4.19
181–82	R. P. 880, phytin 35.7	21.2	6.07	5.63
183–84	R. P. 1320, phytin 35.7	21.6	6.22	6.88

in a week there was a fine stand.   In a few days most of the plants in the pots containing alfalfa or leached alfalfa had died or were severely injured. On February 4 these pots were replanted and the second time developed without any further noticeable difficulty.

Table 4 gives the treatments and yields of the oats on the singly treated series. It will be seen that for each increment of phosphorus added, there is an increase in the yield of grain until the maximum is reached. The alfalfa series produced better than the phytin in the lower treatments, but dropped off in the higher treatments.

Table 5 gives the treatments and yields of the oats on the combination series. It will be noted that in the lower treatments there is a tendency for the yields to be greater than the theoretical yields, which are obtained by taking the sum of the yields for the separate treatments in table 4.

SUMMARY

1. Phosphorus supplied in phytin was found to be more readily assimilated by growing plants than that in the inorganic form.

2. An increase in the rate of application increased the phosphorus content of both the grain and straw of oats, but to a greater extent of the straw. This was found to be true with both organic and inorganic phosphates.

3. A marked toleration of phosphorus was found in the straw of the oats and an indication of some tolerance of that element in the grain.

4. Large amounts of phosphorus as phytin were found to be deleterious to the growing plant.

5. Oats planted 23 days after the addition of alfalfa and leached alfalfa to sand were injured or killed, whereas those planted 14 days later in the same treatments grew normally.

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PLATE 1

RELATIVE GROWTH OF OATS WITH ROCK PHOSPHATE AND CRUDE PHYTIN
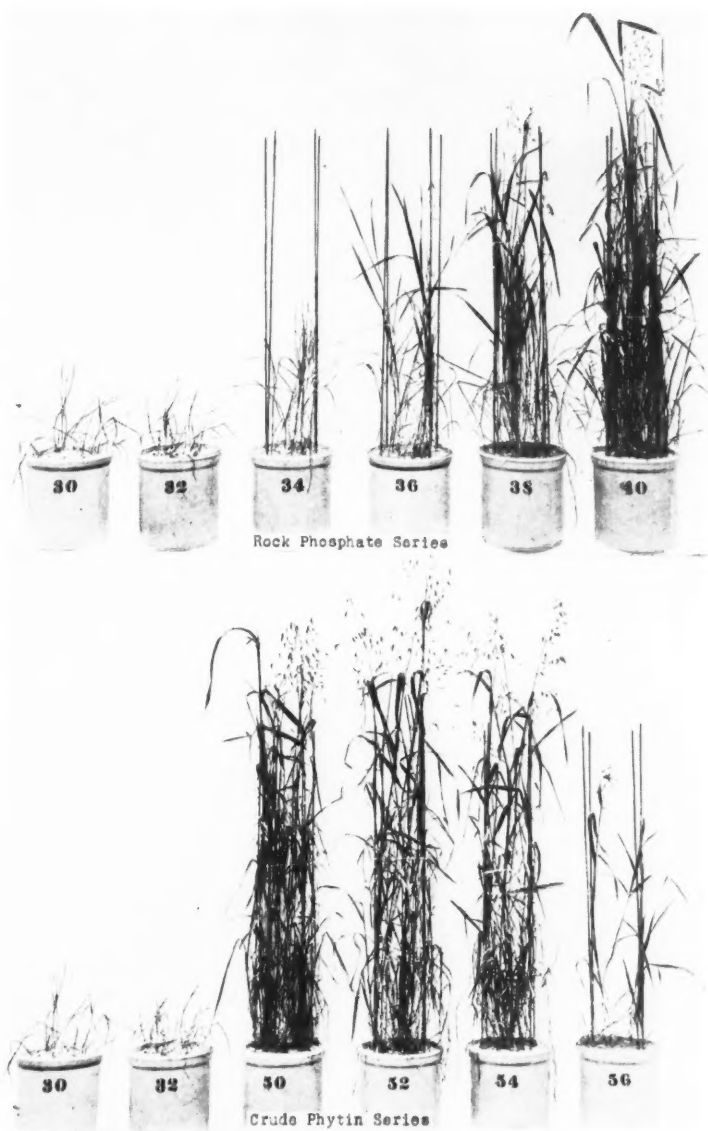
PLATE 2

Above: Pot 101.　 No treatment
　　　　 Pot 104.　 All plant-food except P
　　　　 Pot 114.　　 220 mgm. P per pot
　　　　 Pot 116.　　 440 mgm. P per pot
　　　　 Pot 118.　　 880 mgm. P per pot
　　　　 Pot 120.　 1320 mgm. P per pot
Below: Pot 101.　 No treatment
　　　　 Pot 104.　 All plant-food except P
　　　　 Pot 178.　　 220 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot
　　　　 Pot 180.　　 440 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot
　　　　 Pot 182.　　 880 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot
　　　　 Pot 184.　 1320 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot
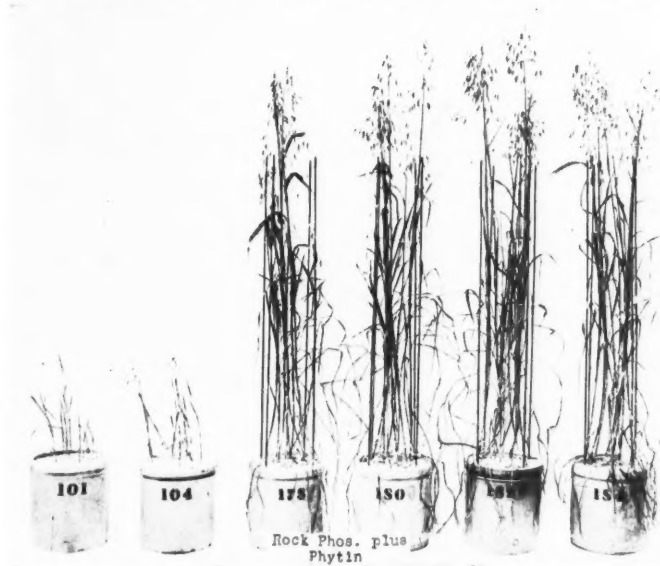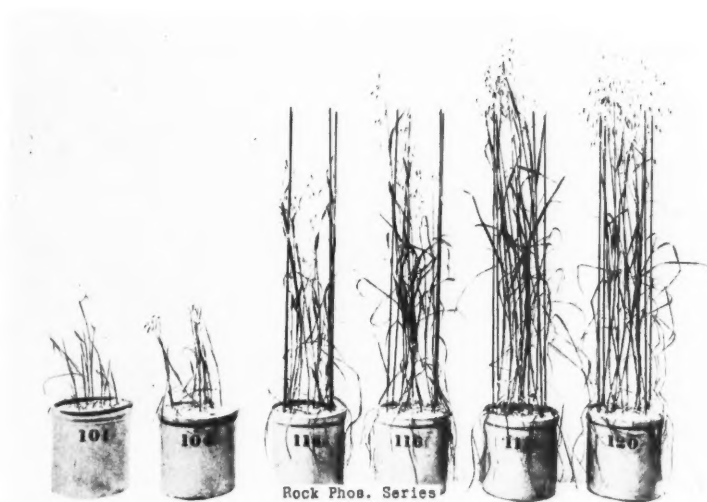
OAT SERIES WITH ROCK PHOSPHATE AND ROCK PHOSPHATE PLUS PHYTIN

Above: Pot 101. No treatment
　　　　 Pot 104. All plant-food except P
　　　　 Pot 138. 11.9 mgm. P per pot
　　　　 Pot 140. 23.8 mgm. P per pot
　　　　 Pot 142. 35.7 mgm. P per pot
　　　　 Pot 144. 47.6 mgm. P per pot
Below: Pot 101. No treatment
　　　　 Pot 104. All plant-food except P
　　　　 Pot 162. 880 mgm. P in rock phosphate + 11.9 mgm. P in phytin per pot
　　　　 Pot 164. 880 mgm. P in rock phosphate + 23.8 mgm. P in phytin per pot
　　　　 Pot 166. 880 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot
　　　　 Pot 167. 880 mgm. P in rock phosphate + 47.6 mgm. P in phytin per pot

A. L. WHITING AND H. F. HECK
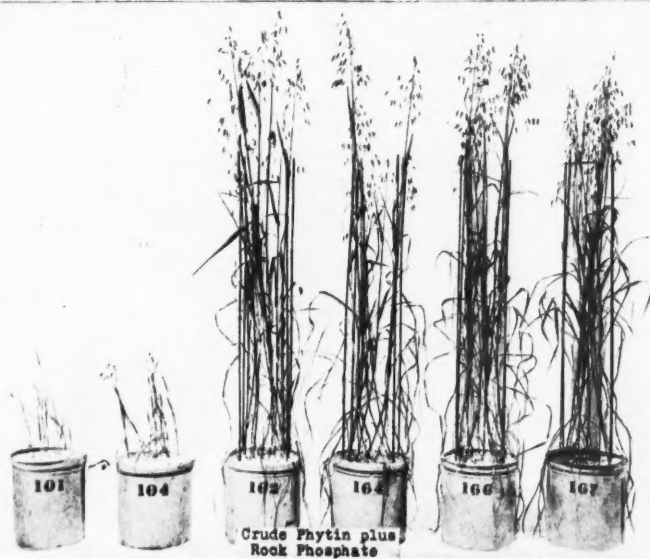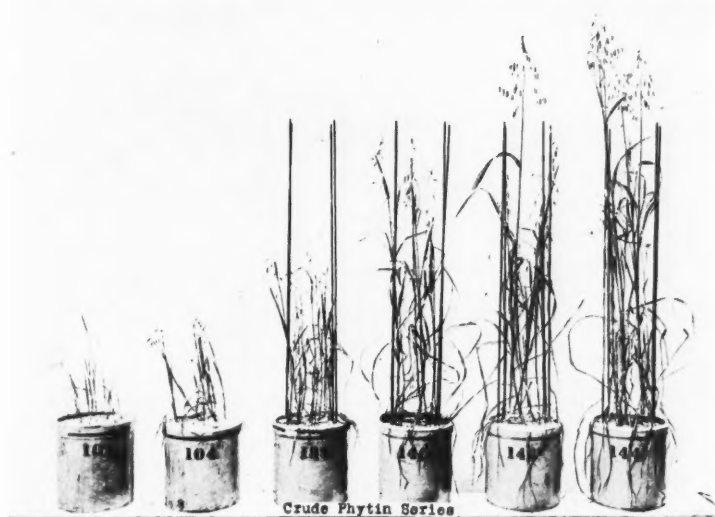
OAT SERIES WITH CRUDE PHYTIN AND CRUDE PHYTIN PLUS ROCK PHOSPHATE

PLATE 4

The figures show the number of milligrams of phosphorus added in the forms indicated.
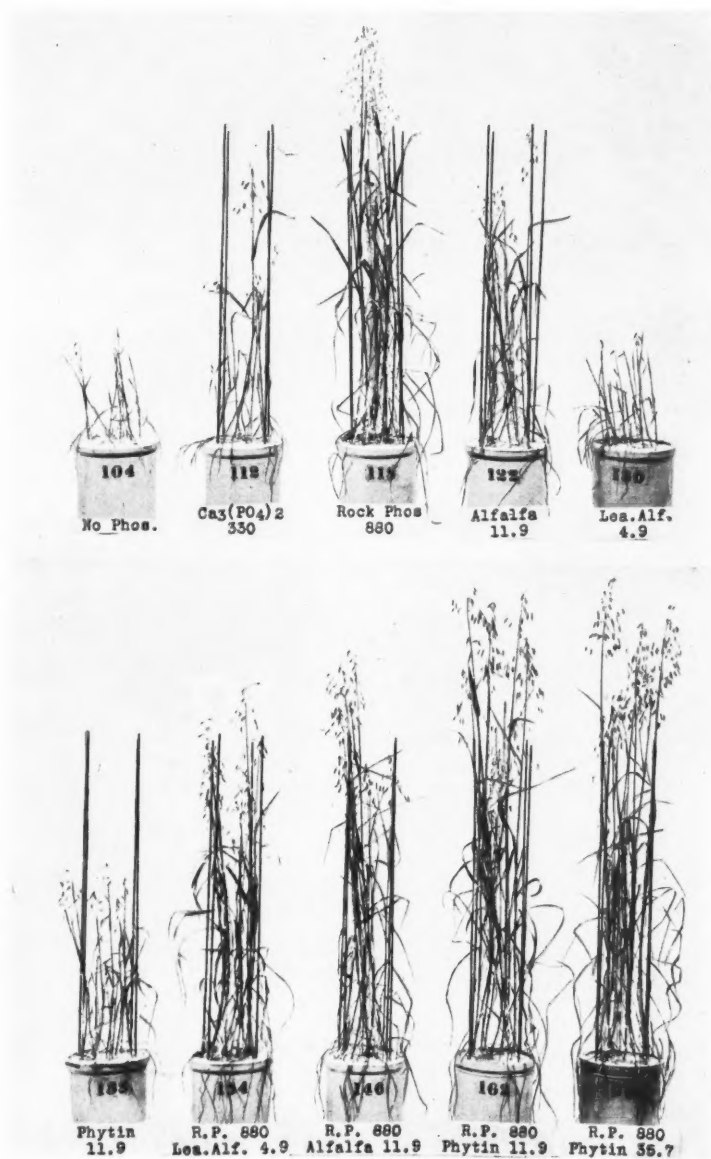
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